GLUCOSE METABOLISM IN OBSTRUCTIVE JAUNDICE

George Michael Flannigan

Ch M

University of Edinburgh

1986

DECLARATION

This thesis was composed entirely by me, and apart from some assistance with the complex biochemical assays it was conducted entirely by me in the Department of Surgery, Royal Postgraduate Medical School, Hammersmith Hopsital, Ducane Road, London, between September 1981 and February 1983.

G Michael Flannigan

"On resisite a l'invasion des armées; on
ne resiste pas a l'invasion des idées."

Victor Hugo (1802-1885).

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ABSTRACT

It has been shown that impaired preoperative nutritional status and the hyperbilirubinaemia of biliary obstruction are associated and are of prognostic importance following surgery. This study examined the relationship between hepatic glucose production and muscle catabolism as demonstrated by the 'Glucose-Alanine Cycle' to assess the nutritional importance of this cycle in patients with biliary obstruction. Twenty three patients were studied, 10 with normal liver function and 13 with obstructive jaundice. All patients were suffering from gastrointestinal or intra-abdominal malignancy. Fasted patients were challenged with an intravenous bolus of alanine and glucose on separate days, both before and after surgery. Nutritional status was comprehensively assessed before each test. No abnormalities in either glucose or alanine metabolism was found which could be associated with the presence of biliary obstruction. No association could be found between variations in nutritional status and alanine or glucose metabolism. Prior to surgery, maximal nutritional support was offered by the method most appropriate to each patient. The postoperative return of serum bilirubin levels towards normal was accompanied by no demonstrable alteration to glucose or alanine metabolism. This study was unable to demonstrate any abnormality in the dynamic equilibrium of the glucose alanine cycle which could be associated with poor nutritional status or biliary obstruction, both of which are related to postoperative prognosis. It does not indicate any method for augmenting routine nutritional support which in turn might improve the postoperative prognosis in patients with biliary obstruction.

PREFACE

1.1.1 Preface

1.1.1

The importance of nutritonal status in the patient with biliary obstruction has been documented by earlier workers in this department (Allison et al 1979, Halliday et al 1982, 1985). On the basis of results from these and other studies (Braasch and Gray 1977, Warren et al 1975) a link between poor nutritional status, the severity of biliary obstruction and high postoperative morbidity and mortality rates is now well established. However, trying to assess the value of nutritional support, particularly in the preoperative period, is difficult.

The increased surgical risk encountered by the patient with obstructive jaundice is multifactorial in nature. In addition to nutritional status being an important prognostic factor (Allison et al 1979, Warren et al 1975) duration and depth of jaundice are important as are related pathologies such as malignancy or the development of renal failure. The question to be answered by this study was, 'Can standard methods of nutritional support be altered or augmented to improve the postoperative prognosis for patients with obstructive jaundice'.

The indications for and value of nutritional support in disease can be studied in three different ways. The first 2 methods are aimed at demonstrating the value, or potential value, of aggressive nutritional support. In the first, indices of nutritional status can be recorded before and after a period of nutritional support. Many

have anticipated that a demonstrable improvement in these indices could be expected to be rewarded by an improved postoperative recovery.

A second method of study, often combined with the first, involves the recording of postoperative morbidity and mortality rates in a group of randomized patients, some of whom have received nutritional support the remainder receiving no additional nourishment. For statistical significance to be achieved in such studies the number of patients admitted to the study would need to exceed 100. Indeed in a recent report (Muller et al 1982) where preoperative feeding in 160 patients was studied a claimed significant reduction in the postoperative morbidity rate was incorrect on re-analysis of the data presented, and the addition of one extra death to the pre-operatively fed group also abolished the significance of the mortality rate. Therefore, even a carefully controlled study involving large numbers of patients fails to provide convincing evidence of the value of preoperative intravenous nutrition over an extended period.

A third approach was suggested for this study. Abnormalities in metabolism at a cellular level might provide the reasons for the particular nutritional deficiences seen in patients with biliary obstruction. The 'Glucose-Alanine Cycle' has been used recently to provide an explanation for nutritional irregularities in septic postoperative patients (Royle and Kettlewell 1981). Since the main organs involved in this cycle are the liver parenchyma and peripheral muscle, it was felt that this would be a useful metabolic process to study in biliary obstruction.

Although abnormalities in the glucose alanine cycle had been previously demonstrated only in parenchymal liver damage, the links already described between biliary obstruction and nutritional deficit were considered sufficiently strong to warrant investigation of this metabolic cycle in obstructive jaundice.

The aim of this study, therefore, was to examine the glucose alanine cycle in an attempt to identify an abnormality specific to biliary obstruction which might suggest appropriate therapeutic counter measures.

Although poor nutritional status is an important prognostic factor in obstructive jaundice (Halliday et al 1982, 1985) two mechanisms may account for this. Muscle wasting and weight loss may be a reflection of the nature of the pathology, (e.g. sepsis from chronic intermittent cholangitis in relation to a ductal calculus or benign stricture), or increased protein turnover, (e.g. a wasting disease like carcinoma of the pancreas). Poor nutritional status in such patients would become an additional burden in the post-operative period as it would in a non-jaundiced patient. Alternatively, the patient with obstructive jaundice might experience an abnormal metabolic drive, resulting in an inappropriate manipulation of endogenous fat, glycogen or protein stores, or an abnormal assimilation of an enteral or parenteral diet. Abnormal fat absorption is well recognized in obstructive jaundice due to the absence of bile salts from the small bowel, and the loss of the entero-hepatic circulation.

The aminoacid alanine is the major protein-derived substrate for hepatic gluconeogenesis. An abnormality in alanine metabolism might

indicate an altered rate of muscle catabolism, or hepatic function, or both. It was the purpose of this study to determine whether patients with obstructive jaundice experienced abnormalities in alanine metabolism in the presence of normal glucose metabolism in either the preoperative or postoperative periods. Such abnormalities might render such individuals more prone to a deterioration in nutritional status with the resultant increased postoperative morbidity and mortality rates. If this was confirmed it might indicate the need for appropriate dietary or metabolic manipulation to improve postoperative prognosis. The absence of such changes would indicate that the routine indications for, and techniques of, nutritional support would need no adaptation for the patient with obstructive jaundice, within the limits of this study.

The clearance of intravenous loads of alanine and glucose was examined. Glucose clearance was measured to determine whether this was normal or abnormal in obstructive jaundice. An abnormal glucose clearance rate would complicate interpretation of alanine clearance studies. In order to select a study group likely to have poor nutritional status only patients with intra-abdominal or gastro-intestinal malignancy were admitted to the study.

Intravenous glucose clearance was impaired in both study and control groups to the same extent. As will be seen later many workers have noted abnormal glucose metabolism associated with malignancy. The presence of obstructive jaundice did not seem to influence this. This is an important finding, as abnormal liver function has also been shown to cause alterations in glucose metabolism.

In addition, it was found that alanine clearance was the same in both groups preoperatively, although there was an implication that those jaundiced patients with clearance rates at the lower end of the range had improved clearance after surgery when liver function had begun to recover.

However, it would appear that obstructive jaundice does not influence the dynamic equilibrium of the glucose-alanine cycle. Equally important was the similarity found in fasting levels of both alanine and glucose in the study and control groups. It implied that the metabolically stable, fasted patient, displayed a glucose and alanine homeostasis unaltered by the presence of obstructive jaundice. Although these are the results of major importance in this study, futher data analysis produced findings of interest which are detailed later.

Glucose metabolism and maintenance of a normal equilibrium in the related processes of hepatic gluconeogenesis and muscle catabolism, appear to be governed by homeostatic mechanisms easily capable of overcoming the abnormal metabolic stresses found in the patient with obstructive jaundice. This suggests that the rate of deterioration in nutritional status in patients with obstructive jaundice may be no worse than would be found in similar pathology in the non-jaundiced patient.

This work represents a rudimentary investigation into amino acid metabolism in the patient with obstructive jaundice in an attempt to determine the role of preoperative nutritional support. There is no evidence from this study to suggest that anything other than orthodox preoperative nutritional support is needed in the patient with

obstructive jaundice. However, other results suggest there may be an indication to prolong or intensify the nutritional support offered postoperatively.

Introduction

"It takes a great deal of history
to produce a little literature."
Henry James (1879).

2.0 INTRODUCTION

2.1.1 Nutrition and the patient with obstructive jaundice

Definitive management of biliary obstruction often presents a major surgical challenge. That it is fraught with high morbidity and mortality rates may have as much to do with abnormal hepatic metabolism as with the complexity of the surgery. Many reports exist of the complication and death rates following such surgery (Braasch and Gray 1977, Warren et al 1975, Fish and Cleveland 1964, Hicks and Brooks 1971, Monge et al 1964, Pliam and Re Mine 1975). Suggested prognostic factors include operative technique, (Hicks and Brooks 1971), attention to blood volume and electrolyte balance (Warren et al 1975), and preoperative biliary drainage (Braasch and Gray 1977, Fish and Cleveland 1964). In addition, the degree of impairment of hepatic function is important. The postoperative complication rate appears to be directly proportional to the preoperative depth of jaundice and preoperative hypoproteinaemia (Braasch and Gray 1977, Fish and Cleveland 1964). In particular hypoalbuminaemia, and a prolonged prothrombin time and poor nutritional status are important prognostic factors (Braasch and Gray 1977, Warren et al 1975, Allison et al 1979).

In the patient receiving surgery for obstructive jaundice, therefore, a combination of meticulous operative and perioperative care, and attention to the metabolic capacity of the liver may reduce to a minimum the postoperative complications (Braasch and Gray 1977). Simple metabolic tests have been used in such patients, in the past,to identify impaired metabolism. Antipyrine clearance (MacPherson et al

1981, Vessell 1979) provides an indirect measure of cytochrome p450 activity in hepatocytes.

The present study was designed to investigate whether the association between impaired preoperative nutritional status and the high morbidity and mortality rates found after surgery for biliary obstruction could be better understood by examining the dynamics of the glucose alanine cycle.

2.1.2 The relevance of nutrition to surgery

In a series of elegant studies on the traumatised patient Cuthbertson (1929, 1930, 1931, 1932) recorded the alterations in body composition, including loss of fat and protein mass, in response to trauma. In addition, he recorded the beneficial effect of a high protein, high calorie diet in halting or reversing this trend (Cuthbertson 1936). In patients receiving surgery for peptic ulcer, Studley (1936) noted a postoperative mortality rate of 33 percent in those who had lost more than 20 percent of their normal body weight. This compared to 3.5 percent in patients with more modest weight loss. Lawson in 1965 examined a group of surgical patients with severe postoperative complications. He concluded that patients who had lost 30 percent of their normal weight at surgery were unlikely to survive. All 8 patients in his study with this degree of weight loss died.

Although the early studies of Studley and Cuthbertson demonstrated an association between poor nutritional status and increased postperative morbidity and mortality rates, an association

between preoperative intravenous nutrition and an improvement in these statistics has been more difficult to demonstrate. Russell and Jeejeebhoy (1983) and Baker et al (1982) have suggested that in suitably controlled prospective studies, the changes in nutritional status are too subtle for accurate measurement and that clinical judgement can be equally useful. Indeed, only two studies (Heatley et al 1979, Muller et al 1982) have suggested preoperative parenteral nutritional support may reduce postoperative complications and sepsis. The most convincing of these (Muller et al 1982) involved studying 125 patients and assessing the value of 2 weeks of preoperative intravenous feeding. A significant reduction in morbidity and mortality rates was reported in the nourished group. However, re-analysis of their morbidity results, using their reported data and the Chi-squared test for significance, demonstrated no siginificant difference between the groups. This was in direct contradiction to the reported results. The addition of one extra death to the mortality group also abolished the statistical significance of this figure. Both statistics had been reported significant for $p < 0.05$ on the Chi-Squared test. Koretz (1984) notes in a personal communication from J Muller that the study was begun with 3 cohorts one of which was subsequently excluded. No statistical correction was made for the inevitable bias produced by this.

Fifteen other studies have been conducted to investigate the value of preoperative and perioperative nutritional support. The pathologies under study include gastrointestinal cancer (Holter and Fischer 1977, Thompson et al 1981), upper gastrointestinal cancer (Moghissi et al 1977, Simms et al 1980, Schildt et al 1981, Lim et al 1981, Moghissi et al 1982), colorectal surgery (Collins et al 1978,

Preshaw et al 1979, Jensen 1982), cystectomy (Hensle 1978, Simms and Smith 1981), ear, nose and throat cancer (Sako 1981), surgery for valvular heart disease, (Abel et al 1976), and abdominal surgery (Garden et al 1983). In none of these studies is there a reduction in either morbidity or mortality rates associated with nutritional support.

Heatley et al 1979 demonstrated that preoperative intravenous feeding reduced the rate of wound infections in patients having surgery for upper gastrointestinal malignancy. Therefore of the two studies existing to suggest that preoperative intravenous nutrition has any value in reducing surgical complications, only one has been correctly reported. The success of the second study may be partly due to patient selection. Patients with upper gastrointestinal malignancy often present with progressive dysphagia. Therefore the weight loss they display is in part due to mechanical problems making food ingestion difficult. It can be argued that this special situation is more likely to respond to intravenous support than other pathologies with associated weight loss where there was no mechanical interference with oral intake.

Although such studies assess the important clinical parameters of morbidity and mortality, and the value of preoperative nutrition, two major problems arise. To obtain valid and useful data large numbers of patients are required, and a homogenous patient population with similar nutritional problems is difficult to obtain. Often combined with this type of study an alteration in a variety of sensitive indicators of nutritional status can be assumed to be of prognostic importance during a period of careful nutritional support. However,

there is still no universal agreement as to the most useful anthropometric and biochemical tests for this type of measurement and relatively large numbers of subjects are still required.

An alternative to these types of study, which has been widely practised, examines metabolic parameters in different disease states to discover abnormalities related to nutritional status which might be correctable with appropriate enteral or parenteral support. The study described here used this method to assess complementary aspects of glucose metabolism in the patient with biliary obstruction. All patients examined were suffering from some form of malignant disease. Such a group was selected as it was thought they would be more likely to demonstrate a significant reduction in nutritional status, thus maximally stressing the metabolic cycles under study.

Metabolic studies have been employed in many different disease states to allow appropriate prescribing of nutritional solutions for optimum effect. In respiratory failure careful studies have demonstrated the value of lipid emulsion in improving the respiratory quotient, helping to maintain adequate surfactant content of the alveoli and reducing the fluid load in the circulation.

In hepatic failure it has been noted that there is an alteration to the normal serum amino acid profile, with a relative increase in the concentration of the aromatic amino acids (AAA) and a decrease in the relative concentration of branched chain amino acids (BCAA). This finding has given rise to the theory that the administration of BCAA in preference to AAA may correct the abnormality and be of therapeutic value in this condition.

Although many studies have been carried out to assess the value of BCAA in liver disease, only 6 of these employed a control group. Egberts et al (1981) studied 30 patients without clinically detectable encephalopathy and noted improved results on psychometric testing of those receiving BCAA. Fiaccadori et al (1980), in a similar study, showed no improvement in encephalopathy with the use of BCAA. In the remaining reports Michel et al (1980) showed no difference between treatment groups, Rossi-Fanelli et al (1982) demonstrated an arithmetic improvement in encephalopathy with no clinical advantage in the BCAA treated group and Cerra et al (1982) showed an improved outcome with the use of BCAA. This finding is in conflict with that of Hagenfeldt et al (1981) in a similar study. There is, therefore, no good evidence to support the routine use of BCAA in patients with severe liver disease.

It was thought, therefore, that a study of a metabolic pathway which involved both muscle metabolism and hepatic function could be a useful tool to determine the effect of biliary obstruction upon nutritional status. For this reason the glucose-alanine cycle (Felig 1973) was investigated.

2.2.1 The glucose alanine cycle

In the surgical patient the main aims of nutritional support are to maintain or increase body mass, in particular protein mass, and to offer sufficient nutrients to produce a reduction in the rate of postoperative complications in particular sepsis and poor wound healing.

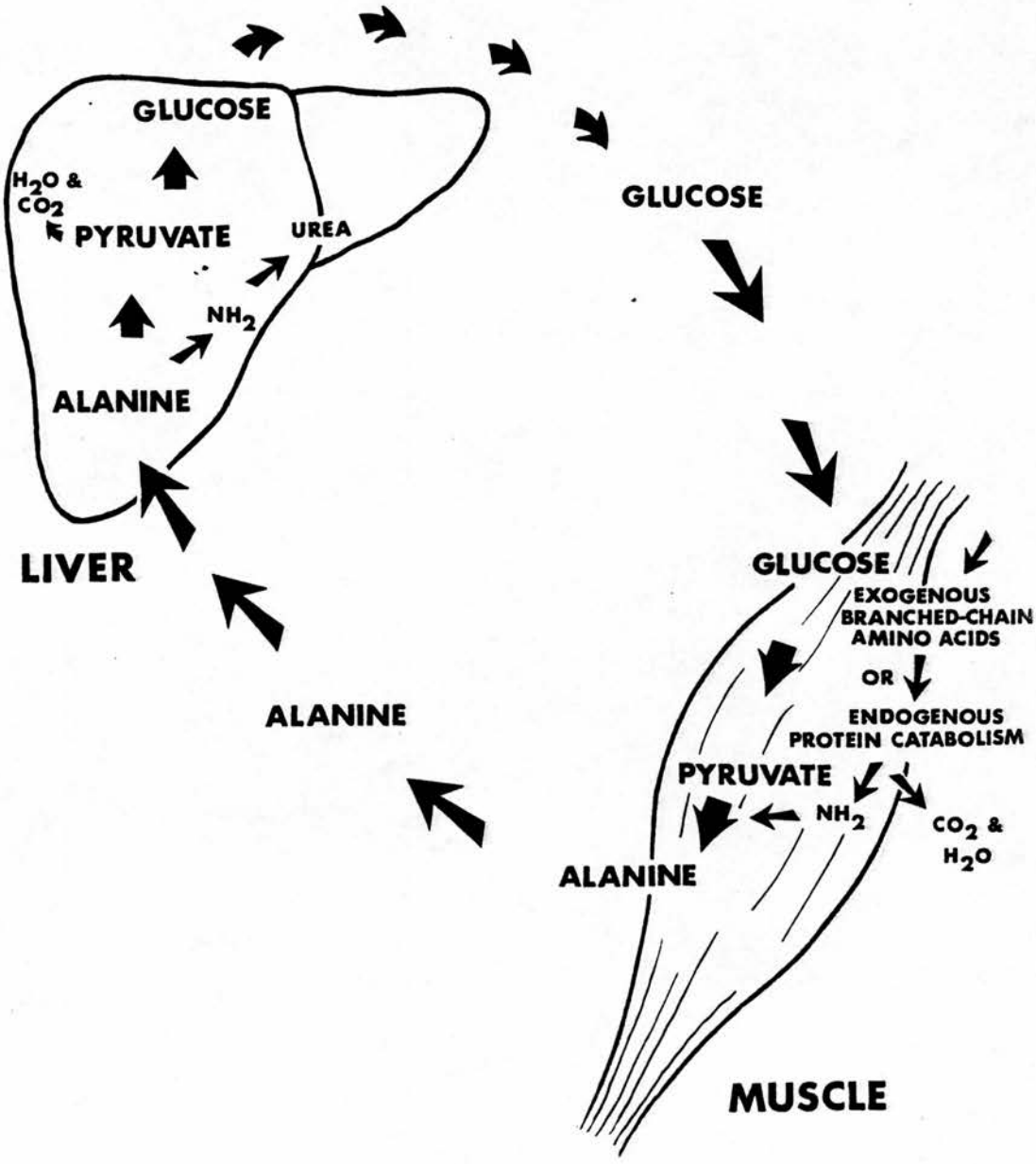


Fig. 1. The Glucose Alanine Cycle

A variety of techniques are available to assess such nutritional status, and most of them measure some some aspect of muscle mass (Bistrian 1981). Peripheral muscle is rapidly catabolised to provide glucose for energy and aminoacids for tissue regeneration and maintenance of immunological competence. Increased muscle catabolism is due to increased energy requirements associated with stress or trauma (Cuthbertson 1930). One of the important metabolic indices of protein catabolism is the rate of alanine release from muscle. Alanine is the principle amino acid produced following muscle catabolism (Felig 1973), the liver being the principle site for alanine clearance.

Unlike carbohydrate and fat, no storage organ exists for protein. All the protein in the body is constantly available for use. It is present as enzymes, contractile protein and structural protein. Excess dietary protein is deaminated, urea being excreted and the carbon framework stored as fat or glycogen.

Cuthbertson (1930) recognised the negative nitrogen balance, increased urinary loss of urea and decreased intake of food associated with injury. Liver glycogen stores are normally exhausted within 24 - 36 hours of fasting (Woolfson 1979). In the patient stressed with injury or sepsis this glycogenolysis occurs more rapidly. The need for glucose, however, continues. The brain is an obligatory consumer of glucose in the early stages of a fast. Although the central nervous system can eventually adapt to use ketone bodies as an alternative energy source, initially glucose must be available. Once glycogen stores are exhausted, glucose is formed from peripheral protein via the pathways of gluconeogenesis.

This process accounts for the majority of muscle wasting in the first days of an otherwise uncomplicated fast. The main products of muscle catabolism are the aminoacids alanine and glutamate. Of these, alanine is the principle gluconeogenic aminoacid being converted to glucose and urea in the liver.

The role of alanine in muscle metabolism and gluconeogenesis was summarised by Felig (1973), (Fig. 1). In a variety of physiological and pathological states, studies of muscle derived alanine and hepatic glugoneogenesis have confirmed the importance of this amino acid (Elia et al 1980, Royle and Kettlewell 1981).

In muscle, pyruvate is transaminated into alanine. The nitrogen radicals which are necessary for this transamination are either products of muscle catabolism or the metabolism of excess dietary aminoacids. The alanine released from muscle, therefore, acts as a carrier molecule for nitrogen radicals and for the carbon skeleton necessary for glucose formation. In the hepatocyte alanine is deaminated, the nitrogen radical converted to urea or transaminated into another amino acid, and the carbon skeleton is converted to glucose. The glucose and amino acids are once more transported to the periphery where the cycle starts once more.

In the fasted individual there is a net release of amino acids from muscle. This was first noted by Van Slyke in 1913 in studies on fasted dogs. The amino acid flux across forearm muscle in the fasted human was studied by London (1965). He recorded that alanine accounted for more than 30 percent of the amino acid released. However, Kominz (1954) noted that alanine accounts for no more than 10 percent of the amino acid residues in skeletal and cardiac muscle.

Therefore there must be a net synthesis of alanine in the muscle in the fasted state. The vast majority of peripheral alanine production arises from the coupling of the carbon skeleton of pyruvate with the nitrogen radicals of the branched chain amino acids valine, leucine and isoleucine.

Many amino acids can be converted to glucose in the liver, but maximal rates for the process are reached for most amino acids at levels 3 times greater than normal. Maximal gluconeogenic rates for alanine, however, are not reached until the blood concentration is at least 20 times normal (Mallette et al 1969).

It would appear, therefore, that a severe impairment of liver function would be required before alanine clearance would be affected. Such a change is an unlikely sequel to biliary obstruction. However, the association between nutritional status and postoperative prognosis in patients with biliary obstruction is well proven (Allison et al 1979, Halliday et al 1982, 1985). The aim of this study was to determine whether the relationship between biliary obstruction and nutritional status could be elucidated with an examination of alanine release from muscle and its clearance in the liver.

Several hypotheses can be proposed. If muscle catabolism is increased in the patient with obstructive jaundice this may be reflected in an increased output of alanine from muscle. Alternatively, an impaired rate of hepatic gluconeogenesis resulting in a reduced or inappropriate muscle protein turnover may reduce the available nitrogen radicals and aminoacids for wound healing and protein reconstruction. Although several workers have shown

abnormalities in this metabolic cycle in a variety of disease states (Elia et al 1980, Royle and Kettlewell 1981), the sensitivity of the methods used and the fundamental relationship between alanine metabolism and nutritional status have yet to be defined.

In the fasted patient ketone bodies appear to limit alanine release from muscle once lipolysis is induced (Royle and Kettlewell 1981). If the blood alanine level rises, however, this inhibits ketone production and the control of muscle catabolism is lost. A failure of alanine clearance might produce such a reaction leading to an increase in muscle catabolism and a subsequent deterioration in nutritional status. However, as already mentioned, except perhaps for terminal, life-threatening stages of biliary obstruction, such a deterioration in liver function is difficult to envisage.

The present study was designed to provide preliminary data in order to help answer these questions. Measuring fasting levels of alanine in patients with obstructive jaundice would offer some evidence of the rate of muscle catabolism. The clearance rate of exogenously administered alanine would determine the integrity of the glucose alanine cycle.

2.2.2 The alanine clearance study

A single measurement of blood alanine in a fasted individual, while offering some indication of the rate of protein breakdown, might be misinterpreted if there was altered hepatic alanine metabolism. The addition of an alanine clearance study would indicate whether the gluconeogenic capacity of the liver had been impaired.

This technique was first used by Felig et al (1969a) when he administered amino acids to fed and fasting subjects. The administration of 10g of alanine or 10g glycine was followed by repeated blood sampling for glucose estimation. No alteration to plasma glucose was noted after administration of either amino acid in fed individuals. After an overnight fast, however, the same individuals demonstrated a marked rise in plasma glucose in response to the alanine load but not to glycine. This is an energy requiring process, since none of the glucose formed is used to fuel the process. Fatty acid oxidation is the principal energy source (Cahill 1970).

The measurment of fasting blood alanine levels in different physiological and pathological conditions can offer only limited information about protein catabolism and the rate of gluconeogenesis. In order to determine the controlling mechanism in gluconeogenesis, and the prevailing flux in the dynamic equilibrium between glucose and alanine, an examination of the rate of hepatic clearance of alanine is required (Elia et al 1980, Royle and Kettlewell 1981). This can indicate whether an alteration in fasting alanine levels is due to hepatic alanine clearance or the release of alanine from the periphery. If normal clearance rates exist the implication would be that any alteration in fasting alanine levels resulted from altered peripheral release.

Since the recognition of the role of alanine in glucose metabolism (Felig 1973) a few workers have employed this dynamic test of hepatic function to help clarify the equilibrium between glucose and alanine in different situations. In order to better understand

the roles of glucagon and insuln in gluconeogenesis, Muller et al (1971) studied the effect of a constant infusion of alanine on fasted and fed mongrel dogs. He concluded that in the fasted state an alanine infusion would cause a rise in plasma glucagon with only a minimal change in insulin concentration. In the fed state, however, when exogenous glucose was available the effect of alanine on these hormones was reversed, the insulin level increasing and the glucagon level remaining unchanged.

In 1974 Fernandes and Blom used alanine clearance studies to investigate inborn errors of carbohydrate metabolism in a group of 8 children. They noted that interpretation of a blood glucose rise following alanine administration may give misleading information about the integrity of gluconeogenesis from this amino acid, as hyperalaninaemia in the fasted individual also promotes glycogenolysis. This may be mediated through the increase in glucagon levels noted by Muller et al (1971). These workers did not suggest the appropriate length of a fast to produce complete depletion of available glycogen, although this is assumed to be 24 -36 hours.

By 1978 specific commoner pathologies were being investigated with this test. Royle & Kettlewell (1978, 1981) noted lower alanine levels in fasted septic patients than in non-septic controls. An alanine clearance study demonstrated unimpaired hepatic gluconeogenesis, suggesting that muscle release of alanine had been reduced in sepsis. It was suggested that a relative hyperketonaemia in the septic patients might be responsible for this decrease in muscle alanine release.

All the patients in the study group had developed sepsis following surgery. All had fasting ketone levels raised significantly above those of the control patients which returned to normal on administration of exogenous alanine. It was suggested that the use of endogenous fat as a principal energy source, with a net release of ketone bodies and resultant hyperketonaemia, afforded control of muscle catabolism and alanine release. That hepatic clearance of alanine remained unchanged indicated that the altered alanine metabolism occurred in the muscle.

The rapid drop in blood ketone body level following the administration of alanine was thought to be mediated through increased insulin production or some non-hormonal mechanism. Nosandini (1981) demonstrated a non-hormonal, direct reciprocal feedback inhibition of alanine on ketone body production. Such a finding is disquieting as it suggests that in conditions where alanine clearance might be impaired as may be anticipated in liver disease, the inhibiting effect of hyperketonaemia might be lost leading to an increased rate of muscle catabolism and consequently of muscle wasting.

Impaired alanine clearance was noted by Elia et al (1980) in patients with hepatic cirrhosis. In that study basal blood alanine and the clearance of an intravenous alanine load was examined in a variety of situations. The patients studied fell into one of several groups, healthy males fasted for 4 days, 4 insulin dependent diabetic patients, 4 patients with hepatic cirrhosis, and 4 patients with muscular dystrophy. In addition, 6 patients having elective total hip replacement were studied before surgery, 6 hours after surgery and 5 and 7 days after surgery.

The fasting blood alanine levels in the surgical, starved and diabetic patients and those with muscular dystrophy were all below normal. Despite the similarity in these results the use of the alanine clearance study demonstrated these findings were due to different mechanisms.

An increased rate of alanine clearance was found after surgery and in the diabetic patients, but it was unaltered in fasted healthy men and appeared to be decreased in cirrhosis, a condition where fasting alanine levels were found to be normal.

Although the control of alanine metabolism occurs in both muscle and liver, peripheral muscle would appear to be the most important site for the control of alanine release (Swaminathan 1981), the metabolic reserve of the liver far exceeding physiological need (Mallette et al 1969).

Recently another controlling mechanism in gluconeogenesis was elucidated. Sacca et al (1983) noted the stimulatory effect of adrenaline on gluconeogenesis from lactate and alanine. Adrenaline appeared to increase the available substrate and had a negligible effect on hepatic clearance. There was a suggestion that exogenous alanine is converted to lactate in the liver and that lactate is a more important gluconeogenic substrate than alanine.

In summary, the glucose alanine cycle is the major metabolic pathway for converting amino acids to glucose. The organs involved in this process are the liver and peripheral muscle. Alanine is released from muscle both following muscle catabolism and following the metabolism of excess dietary protein. Although the capacity of the

liver to clear alanine far exceeds the physiological need, in hepatic insufficiency it is possible that reduced alanine clearance may alter the control of alanine release from muscle. The rate of hepatic gluconeogenesis is therefore of relevance to muscle wasting and nutritional status in the patient with inadequate dietary intake and hepatic failure.

A number of controlling mechanisms have so far been identified. Hyperketonaemia would appear to be the principal inhibitor of muscle alanine production, and a rise in the blood alanine level causes a reflex inhibition of ketone production (Royle and Kettlewell 1981). It follows, therefore, that the induction of hyperalaninaemia in the absence of increased dietary intake of protein would inhibit ketone body production. Such a situation could arise in cirrhosis where reduced alanine clearance has been noted (Elia et al 1980). With the loss of inhibition of alanine release a further acceleration of alanine production will occur with a further decrease in ketone body levels. The presence of an artificial hyperalaninaemia in the patient with liver disease may act as a potent stimulus to muscle wasting and impaired nutritional status. If this situation obtained in the patient with obstructive jaundice it would indicate the need for early institution of adequate nutritional support. However, patients with hepatic cirrhosis and impaired clearance of alanine display normal fasting alanine levels (Elia et al 1980). This suggests that alanine release is still carefully controlled in this pathology.

2.3.1 The intravenous glucose clearance study

As already shown glucose metabolism is linked to alanine metabolism. If alanine metabolism is to be studied in patients with obstructive jaundice more information must be collected about their individual rates of glucose clearance. In order to avoid the complex effects of the hormones released in response to the ingestion of glucose, an intravenous glucose tolerance test would be the most appropriate method to study this. However, interpretation of intravenous glucose clearance has been the subject of much debate in the past, and this is now reviewed.

Since the liver is a major site for glucose clearance, as well as gluconeogenesis, studies of glucose metabolism in liver disease would be inadequate without a glucose tolerance test. Glucose tolerance can be abnormal in a number of conditions. Included among these are malnutrition (Smith et al 1975), bed rest (Lipman et al 1970 and 1972), starvation (Genuth 1966, Cahill et al 1966) and sepsis (Gump et al 1974). Even an individual's diet in the pretest period may be important. West (1978) noted that a high fat, low carbohydrate diet immediately before the test may impair glucose tolerance.

Although glucose clearance is influenced by a number of factors, it is principally the insulin response to a glucose load which will determine the rate of glucose clearance. However, it has been shown recently (Elahi et al 1981), in healthy male volunteers, that there may be a discrepancy between the clearance of an intravenous and oral glucose load in the same individuals. After oral administration of glucose the circulatory levels of gastric inhibitory polypeptide (GIP)

have been noted to increase over similar time courses to the increases in insulin levels. It was shown that patients whose clearance of an intravenous glucose load was impaired, when compared to the clearance of an oral load, had higher basal circulating GIP levels and lower immunoreactive insulin (IRI) responses to intravenous glucose alone. In addition, the response of both these hormones to oral glucose was enhanced, suggesting complementary roles for these two hormones in glucose homeostasis following ingestion of glucose. Such synergy would appear to be lost when glucose is administered parenterally.

The interpretation of intravenous glucose clearance would appear equally complex despite the elimination of gut stimulation (Franckson et al 1962, Moorehouse et al 1964a&b). However, insulin appears to play a major role in the clearance of an intravenous glucose infusion (Samols and Marks 1965).

In summary, therefore, since the mechanisms for clearance of an oral and intravenous glucose load are complex and incompletely understood, the examination of glucose clearance in surgical patients might best be conducted with an intravenous glucose tolerance test as this would avoid the complications of a gastrointestinal endocrine response. In addition, since such patients are likely to receive large quantities of parenteral glucose, as part of their management during a period of nutritional support, the clearance of parenteral glucose is of practical importance.

Both intravenous and oral glucose tolerance studies have been employed in a number of situations to determine the effect and importance of glucose in different physiological and pathological conditions. A review of glucose tolerance in malignancy and in

different hepatic pathologies is therefore necessary.

2.3.2 Glucose metabolism in malignancy

One of the most common situations in which glucose may be administered, as part of nutritional management program, is in the patient with cancer cachexia. Abnormalities of glucose metabolism in this situation may not only indicate what precautions are necessary in administering glucose to such patients, but may also offer some insight into the metabolic abnormalities found in the cachectic patient.

It has been recognised for almost 100 years that malignancy may be associated with impaired glucose tolerance (Freund 1885). In the early part of this century a number of studies focussed on this phenomenon (Friedenwald and Grove 1920) principally as a means to early detection of malignancy. Edwards (1919) accepted a normal glucose tolerance test as strong evidence for the exclusion of a diagnosis of cancer.

However, few held this belief, and in recent years some extra-pancreatic malignancies have been associated with hypoglycaemia (Chandalia and Boshell 1972, Kreisberg and Pennington 1970). A multifactorial aetiology has been thought responsible for this, and although the secretion of insulin-like substances by the tumours has been suggested, (August and Hiatt 1958, Friesen and Miller 1960, Whitney and Heller 1961, Oleesky et al 1962, Steinke et al 1962, Field et al 1963, Floyd et al 1963, Boshell et al 1964, Volpe et al 1965), this has been hotly disputed (Unger 1966, Bower et al 1965).

Hypoglycaemia may also result from decreased lipolysis, decreased gluconeogenesis or massive glucose consumption by the tumour (Butterfield et al 1960, Landau et al 1962).

Such accounts of hypoglycaemia associated with extra-pancreatic malignancy are rare. Much more common are accounts of impaired glucose metabolism in malignancy (Glickman and Rawson 1956, Marks and Bishop 1957, Jasani et al 1978 and Schein et al 1979). Glickman and Rawson (1956) recorded intolerance to orally administered glucose in 36.7% of patients with malignancy as opposed to a 9.3% incidence in those without cancer. This study was carried out on almost 1000 consecutive admissions to the Sloan Kettering Institute.

Schein et al 1979 regularly noted insulin resistance in the patients they studied with malignant disease. Although they could find no abnormality in insulin receptors they noted an increased rate of fatty acid oxidation and gluconeogenesis associated with increased Cori cycle activity. The Cori cycle describes the cyclic metabolic pathway whereby glucose is converted to lactic acid and reconverted to glucose in the liver. The only treatment which seemed capable of correcting these abnormalities involved removal of the tumour.

This insensitivity to insulin was earlier noted by Marks and Bishop (1957). As has already been mentioned, however, many other factors can influence glucose tolerance and it is rare to find a patient with cancer and no history of recumbency or sepsis, starvation or anorexia.

Holroyde et al (1975) examined the rate of glucose production via Cori cycle activity in patients with malignant disease. Patients with

marked malignant cachexia had a rapid glucose turnover and high Cori cycle activity as opposed to patients with malignancy and minimal wasting when Cori cycle activity appeared to approach normal.

It therefore becomes apparent that altered glucose metabolism in association with malignancy is principally found in those patients also displaying marked cachexia.

2.3.3 Glucose metabolism in liver disease

Glucose intolerance has been documented in a number of different liver pathologies since the early part of the century (Coller and Troost 1929, and Althansen et al 1930). In addition to numerous reports of glucose intolerance in hepatic cirrhosis (Kato et al 1973, Samaan et al 1969, Riggio et al 1982, Shankar et al 1983 and Johnston et al 1982b) it has also been found in other hepatic pathologies including chronic active hepatitis (Alberti et al 1972), acute viral hepatitis (Record et al 1973 and Felig et al 1970a), alcohol induced fatty liver (Rehfeld et al 1973), paracetamol induced liver damage (Record et al 1975a) and fulminent hepatic failure (Record et al 1975b). Reports of glucose tolerance in biliary obstruction are less common (Solar et al 1974 and Ozawa et al 1975).

In 22 patients with hepatic cirrhosis Samaan (1969) showed a 50% incidence of intolerance to 50 grams of glucose given orally. He suggested this was due to a higher output of human growth hormone (HGH) than in the patients with normal glucose tolerance. He also noted lower circulating insulin levels in the diabetic as opposed to non-diabetic cirrhotic patients. HGH levels in all cirrhotic patients

were higher than in the normal controls. Riggio et al (1982) recorded impaired glucose tolerance in 13 of 18 patients with cirrhosis of whom 5 displayed frankly diabetic clearance curves. By assessing not only insulin levels but also c-peptide levels, an indirect method for estimating portal insulin concentration (Bonser and Garcia-Webb 1981), it was noted that despite glucose intolerance in cirrhotic patients there was both an increased production of insulin, in response to hyperglycaemia, and a reduced rate of insulin degradation (Johnston et al 1978). Again, high HGH levels were noted in the cirrhotic patients and also high basal free fatty acid levels were found in this group. It was suggested by the authors that high circulating free fatty acid (FFA) levels may be involved in producing the glucose intolerance that was found, although a mechanism was not postulated. In 21 cirrhotic patients studied by Shankar et al (1983) 12 had a normal response to oral glucose, 3 had impaired glucose clearance and 6 had frank diabetes. Again impaired insulin degradation was found in patients with cirrhosis.

In a comparison of patients with cirrhosis and acute viral hepatitis Hernandez et al (1969) recorded a high incidence of glucose intolerance in patients with cirrhosis, 7 out of 9 studied, but a low incidence in acute liver disease, 1 out 5. In a study on 25 patients, by Megyesi et al (1967), it was suggested that insulin resistance and hyperinsulinaemia develop early in liver disease and that this situation may progress to diabetes. However, Hernandez et al (1969) recorded normal IRI levels in all patients with acute liver disease thus, in part, throwing doubt on this hypothesis.

In any study of glucose metabolism in patients with liver

disease, a test of glucose tolerance is necessary to provide important background information for interpretation of further results. Abnormal glucose tolerance can be found in many varieties of liver disease, including biliary obstruction, although it may be more likely if the pathology is chronic in nature.

2.4.1 Nutritional assessment

Nutritional assessment is an essential first step in the management of malnutrition (Blackburn and Bistrian 1976). Controversy surrounds the indications for using any of the available plethora of mechanical, biochemical and clinical investigative techniques available. An appraisal of these techniques and of their indications and value is therefore necessary.

The important early studies of Cuthbertson(1929, 1930, 1931, 1932, 1936) described in detail the alterations of body composition in the injured or stressed patient. His studies noted that the increased losses in nitrogen, sulphur and phosphorous, recordable within the first 48 hours of injury, reached a maximum on the tenth day after injury and thereafter decreased to basal levels. Parallel increases in oxygen consumption, temperature and pulse rate accompanied these metabolic changes. The immobilisation associated with injury was not found to be sufficient cause for these losses and there was continued nitrogen loss in ambulant injured patients, despite muscle atrophy being reduced with movement and massage.

He further demonstrated that pyrexia alone was insufficient stimulus to account for these metabolic losses and concluded that such

changes were part of the body's normal response to trauma forming an appropriate metabolic environment for tissue repair. In later studies he noted the provision of liberal enteral nourishment, with a high content of first class protein, significantly improved nitrogen balance in such patients. In the intervening 50 years between these studies and the present, changes in medical technology and awareness have brought about increasing enthusiasm for nutritional support in the management of the hospitalised patient. The patients studied by Cuthbertson were trauma victims whose gastrointestinal tract was undamaged and functional. A great need existed, however, for intravenous solutions with sufficient nutritional value to sustain life when gastrointestinal function was impaired through trauma or disease. This was apparent in 1936 when Studley recorded a 33.3% mortality, in patients with a greater than 20% weight loss, undergoing surgery for chronic peptic ulcer disease. At that time, there was no means available for adequate postoperative, intravenous nutritional support. Indeed, he concluded that preoperative preparation was of paramount importance in this situation.

Over the last decade there have been many reports of the need for awareness of malnutrition in individuals stressed with illness and surgery. On populations of hospitalized medical (Bistrian et al 1976) and surgical patients (Lee 1980) up to 50% were diagnosed as having protein-calorie malnutrition. Hill et al (1977) noted that a further deterioration in nutritional status can be expected during hospital treatment in those patients already malnourished on admission.

Most studies to determine morbidity and mortality rates in relation to nutritional status employ several methods of assessment.

There are many reasons for this practice. Some patients may be cachectic and display severe muscle wasting, yet owing to accumulating ascites demonstrate weight gain. Therefore the very simple, and usually effective practice, of recording body weight in hospital, as a function of body weight in health, is liable to misinterpretation. Indeed the comparison of present body weight with ideal body weight derived from actuary tables (Metropolitan Life Assurance Tables 1959) which base calculations on a determination of patients height can also be misinterpreted. The situation in which such comparisons are of great value is in an examination for nutritional deficiency in children under the age of 10 years as reported by Waterlow et al (1977). In order to avoid the misinterpretation of such tests a number of other methods for estimating total body protein and fat have been devised. One of the common pitfalls of nutritional assessment, in Western society, is that the majority of patients seen are initially overweight. Excess adipose tissue can give the appearance of a normally nourished state in the presence of significant muscle wasting.

The majority of anthropometric measurements try to circumvent this problem by assessing either the fat content or protein content of the individual. A relatively accurate way to determine the fat content is to measure the skinfold thickness in a number of sites using specially designed skinfold calipers. The technique has been described by Jelliffe (1966). Tables of standard skinfold thickness in a number of sites have been compiled by a number of workers (Seltzer and Mayer 1965, Frisancho 1974, Durnin and Womersley 1974 and Burgert and Anderson 1979). Not only can such measurements on individuals be compared with standard tables but indeed Durnin and

Womersley (1974) related such measurements to an estimate of total body fat content. The estimate of total body fat content was arrived at after weighing a number of volunteers, and estimating total body volume by displacement in water. This allowed a calculation of total body density. From this and a knowledge of the density of adipose tissue an estimate of fat free body mass could be computed.

The limitations in the use of skinfold thickness measurements relate to alterations in body composition with age and sex. As individuals become older there is usually an increase in the ratio of adipose tissue to lean body mass, and women have a higher ratio of fat to muscle. There have also been reports of differences between races (Newman 1956). In addition, unless a rigorous technique is used wide variations in results can occur (Ruiz et al 1971).

$$MAMC = MAC - \pi \times TSF$$

MAMC = Mid arm muscle circumference

MAC = Mid arm circumference

TSF = Triceps skinfold thickness

Fig. 2 Calculation for mid-arm muscle circumference

Not only can a record of skinfold thicknesses give an estimate of total body fat content, but they can also give indirect evidence of muscle content. Such evidence is based on the assumption that the triceps skinfold thickness is approximately twice the average thickness of the skin around the middle of the upper arm, including subcutaneous tissue. By using the calculation in Fig. 2 the "Mid-arm

muscle circumference" can be calculated. The term mid-arm muscle circumference is slightly misleading, and requires careful interpretation, as the measurement also includes the humerus.

A number of workers have prepared tables of norms, for such measurements after studying large numbers of healthy volunteers. (Gurney and Jelliffe 1977, Frisancho 1974 and 1981). The comparison of such figures with those derived from malnourished patients gives an estimate of the degree of muscle loss or wasting in terms of a reduction in arm muscle circumference or cross-sectional arm muscle area. A novel way to estimate arm muscle mass has recently been described (Frank et al 1981) in a very visual manner, by the simple manoeuvre of taking an xray of the upper arm and measuring directly muscle thickness.

In an effort to devise a simpler and more rapid clinical measure of muscle wasting in hospitalized patients or out-patients, Klidjian et al (1980) made extensive use of hand grip dynamometry to estimate not only forearm muscle bulk but also forearm muscle function. It was found that this was very sensitive preoperative test for predicting complications from surgery.

The use of biochemical tests has also been explored as a means to determine nutritional status. The most common test used is measurement of serum albumin. Such a test is of limited usefullness. Albumin has a very large body pool and a relatively long biological half life, approximately 3 weeks. This means that not only does it respond to changes in nutritional status slowly it also responds to nutritional repletion equally slowly. Serum transferrin has also been

studied as its half life of 8 days is slightly shorter. However other factors, including iron deficiency, can affect transferrin levels. The plasma proteins that may have most value are those with the shortest half lives and smallest body pools including thyroxine-binding prealbumin and retinol-binding protein (Ingenbleek et al 1975 and Shetty 1979).

Another measure which incorporates both anthropometric and biochemical data was first described by Viteri in 1970 and that is the creatinine height index. This estimate of nutritional status assumes a uniform and reproducible creatinine excretion per unit of muscle. By referring to tables of creatinine excretion recorded on normal individuals of known height, a reduced creatinine excretion compared with the anticipated figure for normal individuals of the same height would imply that the individual in question had a reduced muscle bulk. Indirect evidence of muscle wasting can therefore be produced. This measurement was used extensively by Bistrian et al (1975) and Blackburn et al (1977).

The problem with all these tests is that they either make such general measurements, as in the case of weight or ideal weight, that they can be seriously misinterpreted, or, they are so specific, as in the case of arm muscle circumference or grip strength, that they cannot to take into account total body protein content.

Commonly the basic intention of nutritional status measurements is to indicate those patients at risk of serious complications from surgery. Impaired nutritional status has been recognised as the commonest cause of secondary immunodeficiency (Chandra et al 1968) which has been shown to lead to increased susceptibility to infection

(Scrimshaw et al 1968, Carney et al 1980, Chandra and Newberne 1977). It must be stressed, however, that these studies reflect the findings in developing countries and relate to paediatric protein calorie malnutrition. The relationship between nutritional status and immunological competence has been investigated by a number of methods (Chandra and Scrimshaw 1980). The value of a variety of delayed hypersensitivity skin test measurements, total blood lymphocyte concentration and the concentration of circulating T-cells have all been used to assess immune responsiveness (Chandra and Schrimshaw 1980, Christou 1979a, 1979b).

In a recent review of the current literature on this subject Dowd and Heatley (1984) conclude that there is only a limited understanding of the effect of nutritional repletion on the avoidance of complications associated with secondary immunodeficiency. Much work is still required in this field.

Two further tests are available which estimate the total body content of a number of different elements. There are considerable data in the literature supporting the relationship between total body potassium and total body nitrogen (Harvey et al 1973). It has long been known that the ratio of potassium to nitrogen content in all soft tissues in both normal and pathological states is constant (Talso et al 1953a, 1953b, 1960). The measurement of total body potassium relies on the occurrence of the very stable radio isotope K^{40} as approximately one atom in every ten thousand of naturally occurring potassium atoms. The amount of K^{40} in any individual can be recorded by means of a total body counting chamber (FIG. 3). From the result an extrapolated figure for total body potassium can be produced

Fig. 3. Total body counting chamber

which takes into account variations in height, weight and age of the individual (Goode and Hawkins 1978).

The use of in vivo neutron activation analysis has gained popularity, in recent years, as a means of assessing body composition (Anderson et al 1964, Hill et al 1978, 1979, Oxby et al 1978, Macfie et al 1981). This technique can produce very convincing evidence to identify the appropriate combination of intravenous solutions for maximal anabolism (Macfie et al 1981).

No matter how attractive such techniques are their routine application to the average clinical situation is uncommon, the main reason being the prohibitive cost of the basic equipment. Many clinical studies which include nutritional status measurements, as part of their protocol, record a large number of anthropometric and biochemical parameters. This is done in an effort to present a comprehensive and balanced account of nutritional status measurements in the individuals being studied, in order to avoid the pitfalls of individual tests (Mullen et al 1979, Maclean 1975, Meakins et al 1977, Kaminski et al 1977, Buzby et al 1980). Baker et al (1982) recently presented data which suggested the assessment of nutritional status based on routine clinical history and examination was as valuable as objective anthropometric and nutrition-related biochemical measurements in the prediction of postoperative morbidity.

Controversy therefore exists between the use of complex anthropometric measurements and clinical observation in determining nutritional status. That they may be equally useful confirms their mutual value as a means of standardising estimates of nutritional status (Baker et al 1982, Russell and Jeejeebhoy 1983).

PATIENTS

"So many gods, so many creeds
So many paths that wind and wind,
While just the art of being kind
Is all the sad world needs."
Ella Wheeler Wilcox (1855-1919).

3.1.1 PATIENTS

This study was approved by the Royal Postgraduate Medical School Ethics Committee in the Hammersmith Hospital. All patients gave fully informed consent to each part of the study.

The 23 patients participating in this study had been admitted to hospital for management of a gastrointestinal or intra-abdominal malignancy (TABLE 1). In 13 the malignant process had caused obstructive jaundice to develop, the remaining 10 having normal bilirubin levels. Obstructive jaundice, in this instance, was diagnosed with a history of dark urine and pale stool, a serum bilirubin level above 100umol/l, an elevated serum alkaline phosphatase level and an abdominal ultrasound examination demonstrating dilated intra-hepatic ducts.

In the jaundiced group there were 7 males and 6 females with ages ranging from 41 to 74 years, while the non-jaundiced, control group of patients comprised 8 males and 2 females whose ages ranged from 22 to 80 years (TABLE 2).

It was planned to study each patient on 2 separate occasions: firstly, on admission to hospital, before any treatment had been offered; and, secondly, on resumption of an enteral diet postoperatively. In addition, in the case of the jaundiced patients, the bilirubin level was required to drop below 100umol/l, or to at least half the preoperative level before the postoperative tests were carried out. In this way it was hoped each patient could act as his own control. For several reasons, discussed later, postoperative studies were possible in only a few patients.

Jaundiced			Non-jaundiced		
Age	Sex	Pathology	Age	Sex	Pathology
74	M	cholangiocarcinoma	75	M	squamous carcinoma of oesophagus
67	M	adenocarcinoma of pancreas	75	M	adenocarcinoma of stomach
59	M	cholangiocarcinoma	81	M	squamous carcinoma of oesophagus
69	M	squamous carcinoma of gall bladder	63	F	spindle cell sarcoma of duodenum
62	M	cholangiocarcinoma of duodenal papilla	22	M	hepatic metastases from adenocarcinoma of colon
53	F	cholangiocarcinoma	71	M	advanced squamous carcinoma of anus
53	F	cholangiocarcinoma	66	M	transitional cell carcinoma of kidney
60	F	cholangiocarcinoma	45	M	hepatic metastases from adenocarcinoma of colon
42	M	adenocarcinoma of pancreas	85	F	adenocarcinoma of colon
67	F	cholangiocarcinoma	46	M	adenocarcinoma of stomach
57	F	adenocarcinma of gall bladder			
60	F	cholangiocarcinoma			
74	M	cholangiocarcinoma			

TABLE 1. Sex, age and relevant pathology of patients in study

	Jaundiced	Non-jaundiced
n	13 (M=7, F=6)	10 (M=8, F=2)
Age range	41-74	22-80
Mean age	60	61

TABLE 2. Summary of patients ages and sexes.

3.2.1 Methods - Introduction

On admission, after the diagnosis had been made, each patient was examined for evidence of nutritional deficit. A number of tests were employed, both anthropometric and serological, to help determine nutritional status.

Included in these measurements were an assessment of weight loss, deviation from ideal weight, skinfold thickness over the triceps, biceps, suprailiac and subscapular areas. This allowed an estimation of fat free mass (Durnin and Womersely 1974) as well as mid arm muscle circumference (Frianscho 1974). Additional measurements of mid forearm muscle circumference and grip strength were carried out as part of a multi-centre study as described by Klidjian et al (1980). In order to provide a broader nutritional assessment, serum albumin, thyroxine binding prealbumin and transferrin were measured and total body potassium estimated.

Creatinine clearance, twenty-four hour urine volume, urinary urea and electrolyte excretion were measured to determine renal function on the day of study. Hepatic function was recorded with routine laboratory estimates of bilirubin, aspartate transaminase and alkaline phosphatase. In no patient was there any evidence of sepsis at the time of study. Additional data collected included the duration of illness and length of post-operative stay.

Glucose and alanine clearance levels were carried out on consecutive days. Patients were randomly offered either a glucose tolerance test or an alanine tolerance test on the first day and the second test on the following day.

3.2.2. Intravenous Glucose Tolerance Test.

After an overnight fast of eight to ten hours, an intravenous cannula was inserted into a large ante-cubital vein and 10ml of blood taken for glucose and free fatty acid (FFA) estimation with part of the sample being stored to allow hormone estimation at a later date.

The samples were anticoagulated in lithium heparin tubes containing 100ul of apoprotenin. 100ul was immediately deproteinised in 1ml of uranyl acetate solution, the remainder being centifuged at 8000 r.p.m. for 15 minutes. The plasma was then divided into approximately 2ml and 6ml portions, the former for FFA estimation and the latter stored at $-15^{o}C$ for later hormone assay.

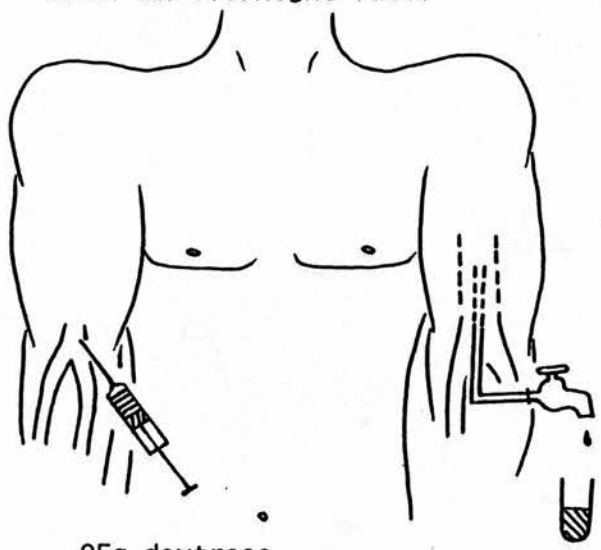
A rapid infusion of 25g of aqueous dextrose solution was then given Winto the opposite arm over a period of 2-3 minutes. The timing of further samples was made from the end of injection. Further blood samples were taken from the indwelling intravenous cannula and following each sample the cannula was flushed with 10ml of normal saline. Prior to each sample being collected 10ml of blood mixed with the residual intra-cannular saline was withdrawn to ensure an undiluted sample was collected. The mixed blood was rapidly returned to the patient before thrombus formed in order to limit blood loss over the test period (Fig. 4).

Following the glucose injection six further 10ml samples were collected in this fashion at ten minute intervals. At the end of the test the intravenous cannula was removed and the wound dressed.

Fig. 4.

INTRAVENOUS GLUCOSE TOLERANCE TEST

After an overnight fast:



25g dextrose
over 3 mins.

Time		Tests
0 mins.		
10		
20		Glucose
30	for	&
40		FFA
50		
60		

10ml at each time point

Glucose was assayed enzymatically (Werner et al 1970) and FFA assayed colorimetrically (Duncombe 1964). All analyses were carried out twice and the average value recorded. The problems encountered in this procedure were related to the FFA assay. It was already known that haemolysis interfered with the analysis, however, once sufficient patients had been studied it became evident that significantly higher readings for FFA levels were being obtained in the jaundiced patients.

Subsequent analysis of FFA levels in normal plasma with varying amounts of bilirubin added, revealed a similar alteration to those found in the plasma of jaundiced patients. Since this problem was discovered toward the end of the study appropriate action to abolish the effect of the bilirubin contamination was not introduced as it could only be applied prospectively to a small number of results. A significant difference between FFA levels in jaundiced and control patients cannot therefore be considered to be real, but the rate of change of FFA levels can still be studied.

3.2.3 Intravenous Alanine Tolerance Test

This test was carried out in a similar fashion to the glucose tolerance already described. After an overnight fast of 8-10 hours an indwelling cannula was inserted into a large forearm or antecubital vein. A 17ml venous blood sample was collected, 8ml being immediately deproteinized in ice cold perchloric acid and 10ml placed in a lithium heparin tube containing 100ul of aprotinin. 100ul was immediately deproteinized in uranyl acetate for glucose estimation.

An aqueous solution containing 12g of L-alanine was then injected intravenously in the opposite arm over 3-5 minutes. Every 10 minutes over the next hour, one further 17ml sample was collected and stored as detailed above. Between each sampling time the intravenous cannula was flushed with 10ml of normal saline. To ensure the collected sample was undiluted, prior to each sample being drawn, 10ml of blood mixed with saline was drawn off and quickly replaced after sampling, before thrombus formed (Fig. 5).

All blood samples were centrifuged immediately at 8000 r.p.m. for 15 minutes and the supernatant underwent enzymatic analysis for glucose (Werner et al 1970), pyruvate (Czok and Lamprecht 1974), D-(-)-3-hydroxybutyrate (Williamson and Mellanby 1974), alanine (Williamson 1974), lactate (Hohorst 1965), and colorimetric analysis for free fatty acids (FFA) (Duncombe 1964).

As with the glucose tolerance test it was discovered at a late stage in the study that the high bilirubin level in the serum of the jaundiced patients interfered with the colorimetric assay for FFA. No correction could be made for this so only a comparison of clearance rate of FFA can be considered significant. Comparison of individual levels is not possible.

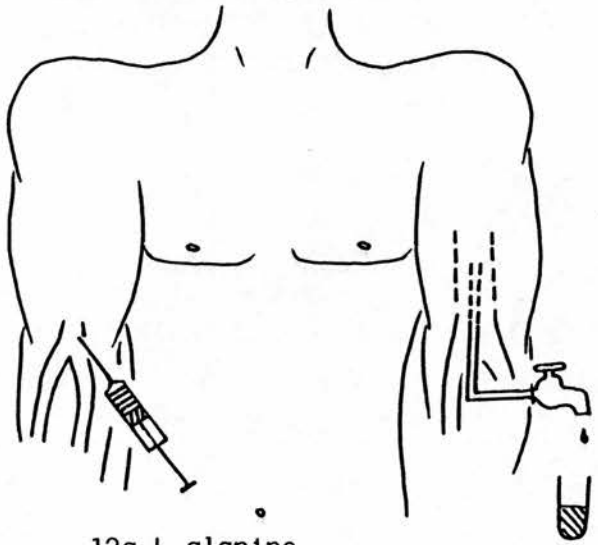
In this test, as in the glucose tolerance test, plasma samples treated with apoprotenin were stored at $-15^{o}C$ for later hormone analysis. At the end of the sampling period the intravenous cannula was removed and the wound dressed.

During administration of the alanine solution some patients initially developed vertigo or felt faint. A reduction in the rate of

Fig. 5.

INTRAVENOUS L-ALANINE TOLERANCE TEST

After an overnight fast:



12g L-alanine
over 3 mins.

Time		Tests
0 mins.		
10		alanine,
20		glucose,
30	for	acetoacetate,
40		pyruvate,
50		lactate,
60		FFA,

17 mls at each time point.

alanine infusion abolished the symptoms, none of which persisted for more than 1 or 2 minutes.

A 12g intravenous dose of alanine was chosen as this is the amount used by other workers (Elia et al 1980, Royle & Kettlewell 1981) thus allowing a direct comparison of this work with theirs. Initially it was considered that this would produce unphysiological levels of alanine in the circulation therefore the first patient studied was offered only 5g of alanine. However, as can be seen in TABLE 3 and Fig. 6, there is little evidence of a change in blood alanine level between the peak level and 1 hour level. Unfortunately the fasting level is missing as it was not suitable for assay.

The remaining 22 patients studied had a mean fasting level of 0.145mmol/l with a range from 0.071-0.291mmol/l. Therefore, although it is likely that this patient's blood alanine level rose in response to the 5g alanine infusion, the rise was minimal and the clearance rate negligible. This patient was not involved in further studies. For these reasons the apparent convention for administering 12g to each patient was adopted. Since this was not offered as a weight related dose, some variation in the peak level was anticipated as result of differing body bulk among the patients studied. It was also found that peak levels were 3 times lower than would be expected after a 12g injection, and did not match those found in previous work (Elia et al 1980, Royle & Kettlewell 1981).

It appears there is a multi-exponential clearance of alanine from the circulation, but 10 minutes after injection of alanine the clearance curve becomes effectively linear. To clarify this point a number of early samples, from 1 minute to 10 minutes, were collected

TIME (Mins)	ALANINE CONC. (mmol/l)
0	-
10	0.57
20	0.50
30	0.36
40	0.45
50	0.51
60	0.45

Table 3. The clearance of 5g of L-alanine

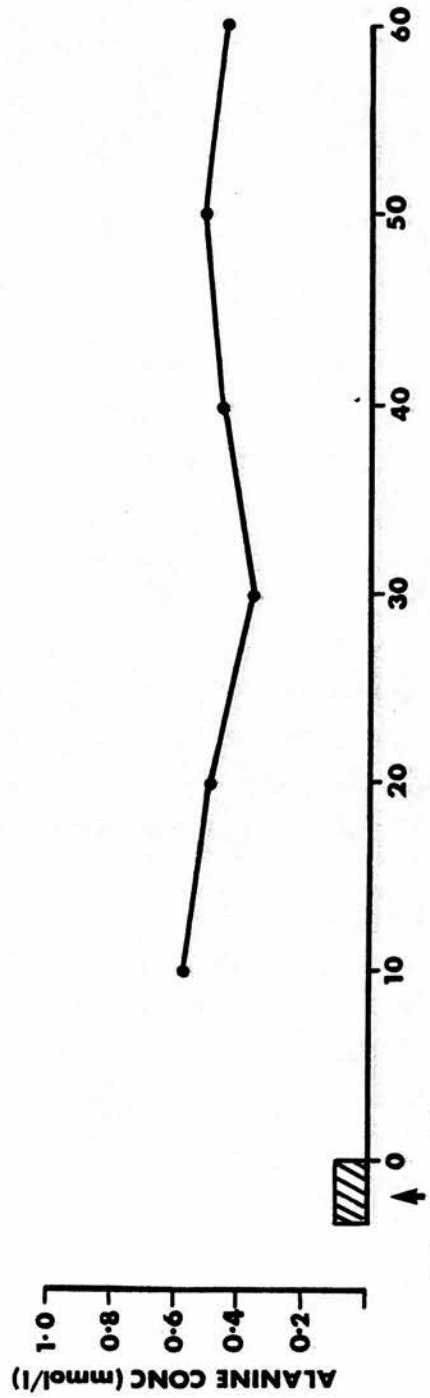


Fig. 6. The clearance of 5g of L-alanine

and the results, TABLE 4 and Fig. 7, demonstrate rapid clearance of alanine and a peak level in keeping with the expected level.

In the earlier studies of Elia et al (1980) and Royle and Kettlewell (1981) the timing of samples started at the beginning of the alanine infusion. For this reason their recorded peak level was higher than is found in this study where timing began at the end of the infusion. Quality testing of random samples of the alanine solution confirmed its concentration at the time of injection to be that calculated by the pharmacy department where the solutions were prepared.

Of the initial 22 patients studied 14 did not undergo postoperative study. Four patients refused consent for further investigation. Six patients did not undergo surgery. One patient died preoperatively and in 3 others bilirubin levels were not sufficiently improved postoperatively, by the time of discharge in 2, and eventual death in the third, to act as reasonable controls. There were only 8 patients in whom any postoperative studies could be carried out.

3.2.4 Anthropometry and nutritional assessment

The nutritional status of each patient admitted to this study was recorded in a variety of ways at the time of both the preoperative and postoperative studies. Measurements of percentage weight loss were made in two ways: firstly, by comparing the patient's measured weight with their reported weight in health; and secondly, by comparing their measured weight with the anticipated weight relative to their height

Time (mins)	Alanine concentration (mmol/l)
0	0.16
1	10.03
2	11.89
3	8.65
4	6.88
6	3.45
8	2.22
10	1.73

Table 4. The early clearance of 12g of L-alanine

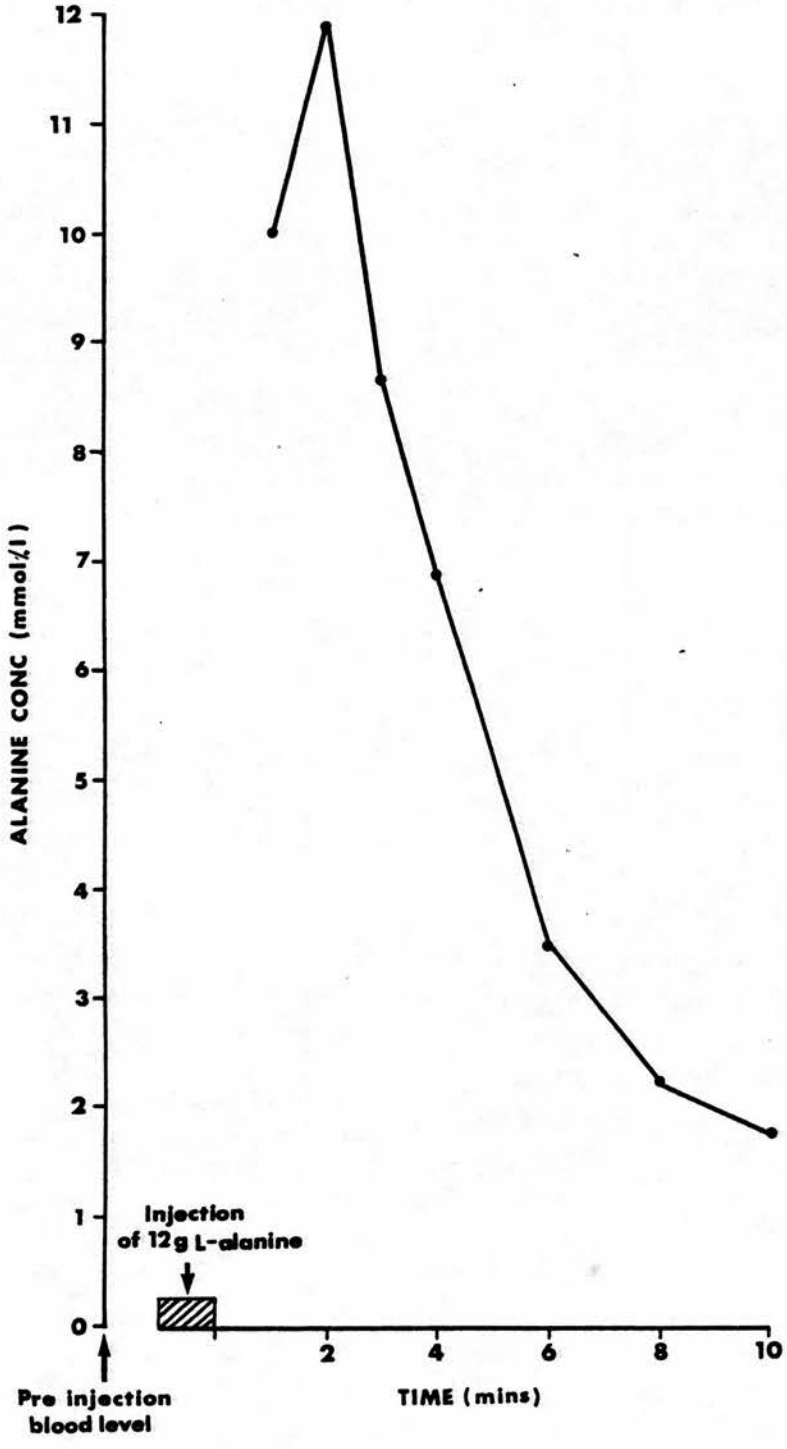


Fig. 7. The early clearance of 12g of L-alanine

(Metropolitan Life Assurance tables 1959). Other physical measurements included grip strength (Klidjian et al 1980) and skinfold thickness (Jelliffe 1966). Using these results an estimate of nutritional status can be made by comparison with tables of normal values (Frisancho 1974). In addition, an estimate of fat free mass (or lean body mass) was made by reference to tables produced by Durnin and Womersley (1974). A blood sample was taken for estimates of plasma albumin, transferrin and thyroxine binding pre-albumin, and when possible the patients total body potassium was calculated (Goode and Hawkins 1978).

3.3.1 Analytical Methods

Owing to the small number of samples in each group under study it was assumed that no population of data formed either a normal distribution or a "t" distribution. For this reason data populations are summarised by using the median as the midpoint of the values and the range to illustrate the spread of data. The Mann Whitney U test was considered the most suitable analytical test for comparing ranges (Siegel 1959).

The clearance curves for alanine and glucose are assessed in a number of ways in order to summarise the data accurately as well as present them in a manner which allows easy comparison with previous studies. Linear regression analysis, using the method of "least squares" (Armitage 1977), was employed to test each substrate curve for linearity and deviation from the X-axis. The "F" distribution (Armitage 1977) was employed to examine the significance of such linearity or deviation. The Chi squared test with Yate's correction for small numbers and Fisher's exact test (Seigel 1959) were used to compare proportional differences.

The clearance rates for both alanine and glucose were expressed in 2 further ways. The substrate half-life was calculated in the standard fashion and its reciprocal (k) the rate constant recorded. Finally, a clearance rate, taking into account the patients weight and substrate distribution volume was calculated (Elia et al 1980).

Graphic representation of data has been introduced to help in its description in certain parts of the text. In order to simplify the drawing of the graphs, while still offering some information as to the

range of values, mean values and standard errors on the mean are represented.

Many calculations, including regression analysis, was carried out with the help of standard spread sheet software running on an Apple II plus* 64K micro-computer.

Accuracy of analytical methods

The percentage substrate recovery was as follows:

alanine	98.5	± 3.2	S.D.
glucose	99	± 4.25	S.D.
lactate	103.5	± 6.3	S.D.
pyruvate	98.7	± 3.75	S.D.
acetoacetate	104	± 5.4	S.D.
FFA	97	± 4.3	S.D.

* - Trademark of the Apple Corporation Inc., Cupertino, California, USA.

RESULTS

4.1.1 Results

Substrate levels in the preoperative studies are recorded in TABLES 6 to 29 all of which can be found in Appendix I. The results for patients with biliary obstruction and non-jaundiced controls are in separate tables. For each set of results a separate summary of regression analysis indicates whether a linear correlation exists between values and if a slope is present. A separate table, following each set of results records comparisons between groups of fasting levels and peak levels or clearance rates for each substrate.

The labelling of each column of results, 1-12 in the jaundiced group and 1-10 in the non-jaundiced group, is specific for a single patient. Not all patients underwent all tests. The tables of results indicate by number which were involved at each stage.

4.2.1 Preoperative alanine tolerance test - Alanine Levels.

The methods used for analysing the clearance of alanine from the circulation have been adapted from those developed for analysis of glucose clearance curves. A full description of the required mathematical transformations appears in section 4.3.1.

In patients with biliary obstruction, fasting levels of alanine ranged from 0.07-0.22mmol/l (Table 6a) with a median of 0.135mmol/l (Table 8), while in the control patients, fasting levels ranged from 0.11-0.29mmol/l (Table 7a) with a median of 0.168mmol (Table 8). There is no significant difference between these ranges of values.

Alanine clearance was calculated in 4 different ways. In all calculations the slope of the curves between the 20 and 60 minute samples was measured to determine clearance rates. Linear regression analysis, of the collected values at each time point, demonstrates a linear relationship between these values (Tables 6b, 7b). However, some workers (Elia et al 1980, Royle and Kettlewell 1981), concerned with exponential nature of the clearance slope, consider a logarithmic transformation of these data allows a more accurate estimate of the clearance rate. Such transformations have been used here and will allow comparisons to be made with the work of others.

With the data represented on semi-logarithmic graph paper the alanine half life can be measured and a rate constant (k) calculated. In addition, a clearance rate which takes into account the alanine distribution volume and individual body weights was calculated (Newton et al 1978). The results of these analyses are summarised in TABLE 8. No differences were found between the clearance rates in the jaundiced and non-jaundiced groups by any method.

A graphic representation of these data can be seen in Fig. 8. Fasting levels, peak levels and slopes appear remarkably similar. The range of values at each time point is represented by the standard error on the mean.

4.2.2 Preoperative Alanine Tolerance Test - Glucose Levels.

Fasting levels of glucose in jaundiced patients and non-jaundiced patients ranged from 2.97-5.44mmol/l (Table 9a) and 3.01-5.50mmol/l (Table 10a), respectively. Median values were 3.635 and 3.71,

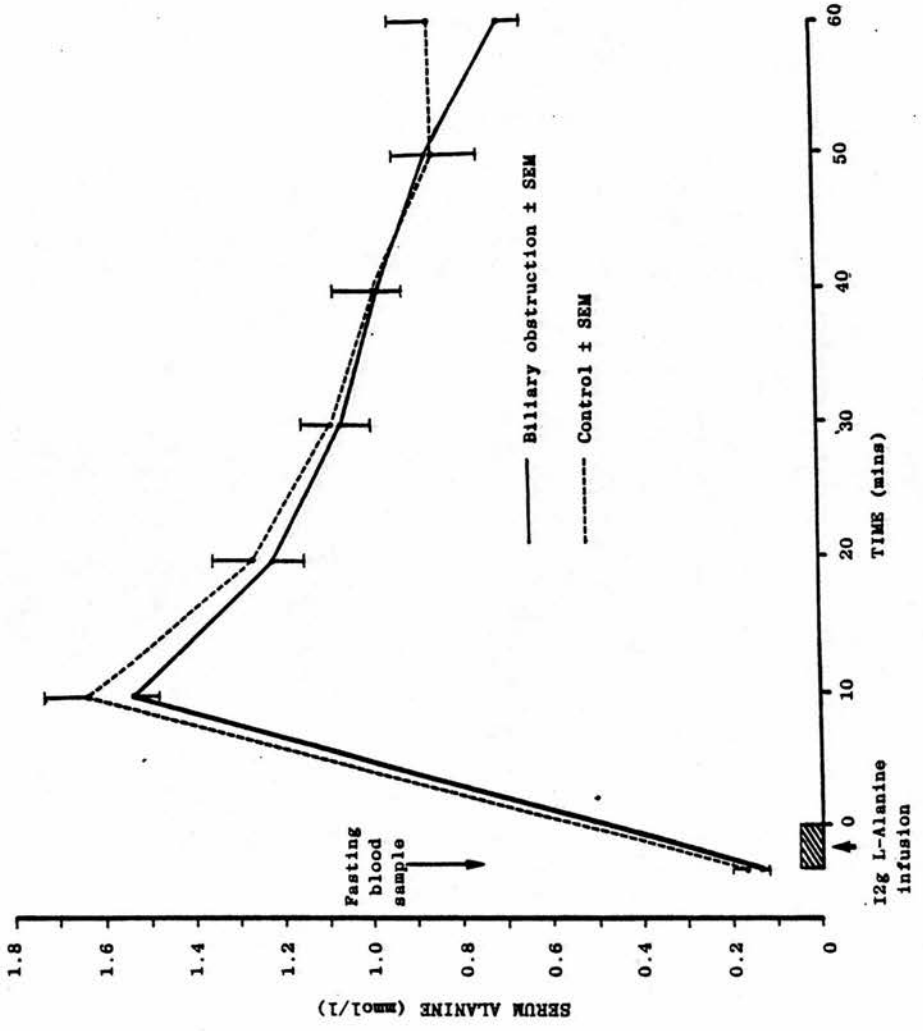


Fig. 8. Preoperative alanine clearance curves

respectively, (Table 11a), there being no significant difference between the groups. Regression analysis revealed that all points conformed to linearity and there was no significant deviation from horizontal, (Tables 9b and 10b), although there appeared to be a slight rise in glucose levels throughout the study with median peak levels being 4.71 and 4.265mmol/l in the jaundiced and non-jaundiced group respectively (Table 11b).

In addition to rise in blood glucose being insignificant, it is noteworthy that the peak levels occurred at different times in each curve. In some, the fasting level was equal to the peak level.

4.2.3 Preoperative Alanine Tolerance Test - Free Fatty Acid (FFA) Levels.

Fasting FFA levels ranged from 0.46-1.32mmol/l (Table 12a) and 0.41-0.97mmol/l (Table 13a) in the group with biliary obstruction and the non-jaundiced group respectively. The median levels in each group were 0.8 and 0.595mmol/l respectively, representing a significant difference between the groups. However, the FFA clearance rate in response to the alanine load ranged from -0.006 to -0.011mmol/min (Table 12a) in the jaundiced group and from 0 to -0.009 in the control group, (Table 13a). Median clearance rates are identical at -0.007mmol/min. This is demonstrated well on the graphic representation of the mean slopes for each group in Fig. 9. Regression analysis, Tables 12b and 13b, confirms the linearity of the values represented and the deviation from horizontal.

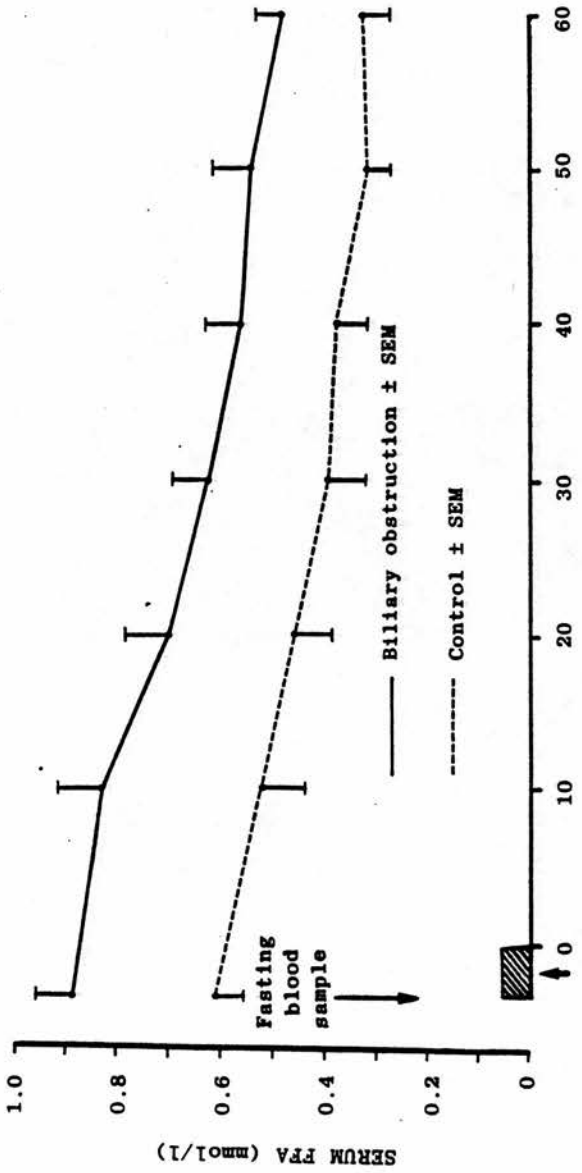


Fig. 9. Preoperative FFA clearance in response to alanine infusion

Fasting levels, however, are significantly different with the median value for patients with biliary obstruction being 0.8mmol/l and that for the control patients being 0.595mmol/l. The reason for this difference has been discussed in section 3.2.3.

4.2.4 Preoperative Alanine Tolerance Test - Lactate Levels

Fasting lactate levels are similar in both groups ranging from 0.27 to 2.31mmol/l in the groups with biliary obstruction (Table 15a), and from 0.35 to 1.38mmol/l in the control group (Table 16a). Median fasting levels were 0.83 and 0.795mmol/l respectively (Table 17a). There is no significant difference between these values. Median peak levels of 1.38 and 1.185mmol/l (Table 17b) imply an increased lactate release throughout the test period. Linear regression analysis (Tables 15b and 16b) suggest that although both sets of data conform to a straight line, there is no significant deviation from the horizontal axis.

4.2.5 Preoperative Alanine Tolerance Test - Pyruvate Levels.

Fasting pyruvate levels are similar in both groups ranging from 0.03 to 0.14mmol/l (Table 18a) in the jaundiced group and 0.03 to 0.16mmol/l in the non-jaundiced group (Table 19a). Median fasting values were 0.109 and 0.094mmol/l respectively (Table 20b). There is no significant difference between these results. Median peak levels of 0.159 and 0.142mmol/l (Table 20b) imply an increased pyruvate release throughout the test. Linear regression analysis (Tables 18b

and 19b) suggests that although both sets of data conform to a straight line there is no significant deviation from the horizontal axis.

4.2.6 Preoperative Alanine Tolerance Test - Acetoacetate Levels.

This assay proved particularly troublesome and many samples were unsuitable for testing. Of the results obtained, fasting acetoacetate levels ranged from 0.05 to 0.14mmol/l (Table 21a) in the jaundiced group, and 0.05 to 0.11mmol/l (Table 22a) in the non-jaundiced group. Median levels were 0.09 and 0.087mmol/l respectively (Table 23a) there being no difference between groups. Peak acetoacetate levels were slightly higher than fasting levels, with median levels of 0.014 and 0.101mmol/l respectively (Table 23b). Linear regression analysis indicated no evidence of a gradient in otherwise straight lines (Tables 21b and 22b). However, ranges of values were very large and differences between fasting and peak levels not significant.

4.3.1 Preoperative Intravenous Glucose Tolerance Test - Glucose Levels.

The intravenous glucose tolerance test was intensively studied as a diagnostic tool between 1930 and 1970 (Althausen et al 1930, Amatuzio et al 1953, Greville 1943, Hamilton and Stein 1942, Lundbaek 1962, Marks and Bishop 1957, Samols and Marks 1965). Such a test can be analysed in a variety of ways. Several methods have been used here to allow comparison of these data with the results of others.

The small populations of subjects in this study, 11 with biliary obstruction and 8 controls, provide too few data for testing with the Student's t test. The results have therefore been expressed in terms of medians and ranges with the Mann Whitney U test being employed to compare ranges.

A direct comparison of the ranges of fasting glucose levels in jaundiced and control patients showed no signficant difference between the groups (TABLE 26). The individual values are recorded in TABLES 24a and 25a.

Analysis of the clearance of the intravenous glucose load is expressed in a variety of ways, similar to the description of alanine clearance, already discussed. Multiple linear regression analysis (Armitage 1977) reveals that the collected clearance slopes between the 20 and 60 minute sample times present a linear relationship which deviates significantly from the horizontal (TABLES 24b and 25b)

A comparison of mean glucose clearance slopes for the jaundiced and non-jaundiced patients reveals no significant difference (TABLE 26 and Fig. 10). However, many workers have preferred to express the clearance rate as a function of the semi-logarithmic plot of the blood glucose values (Hamilton and Stein 1942, Greville 1943, Amatuzio et al 1953, Hlad and Elrick 1959, Lundbaek 1962 and Samols and Marks 1965). This method eliminates or minimises any error due to the slight exponential nature of the curve between the 20 and 60 minute samples.

If the results are plotted on semi-logarithmic graph paper the substrate clearance half-life can be measured directly (Fig. 11). Although the median glucose half-life in the jaundiced group is higher

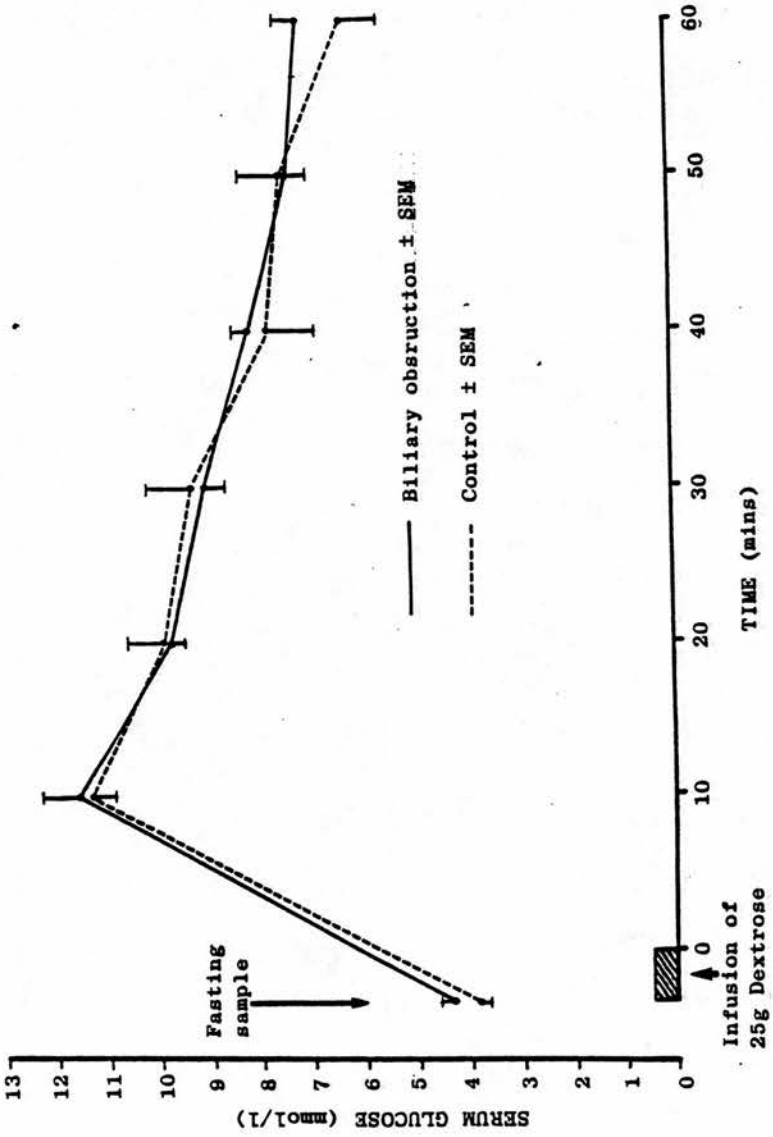


Fig. 10. Glucose clearance after infusion of 25g of glucose

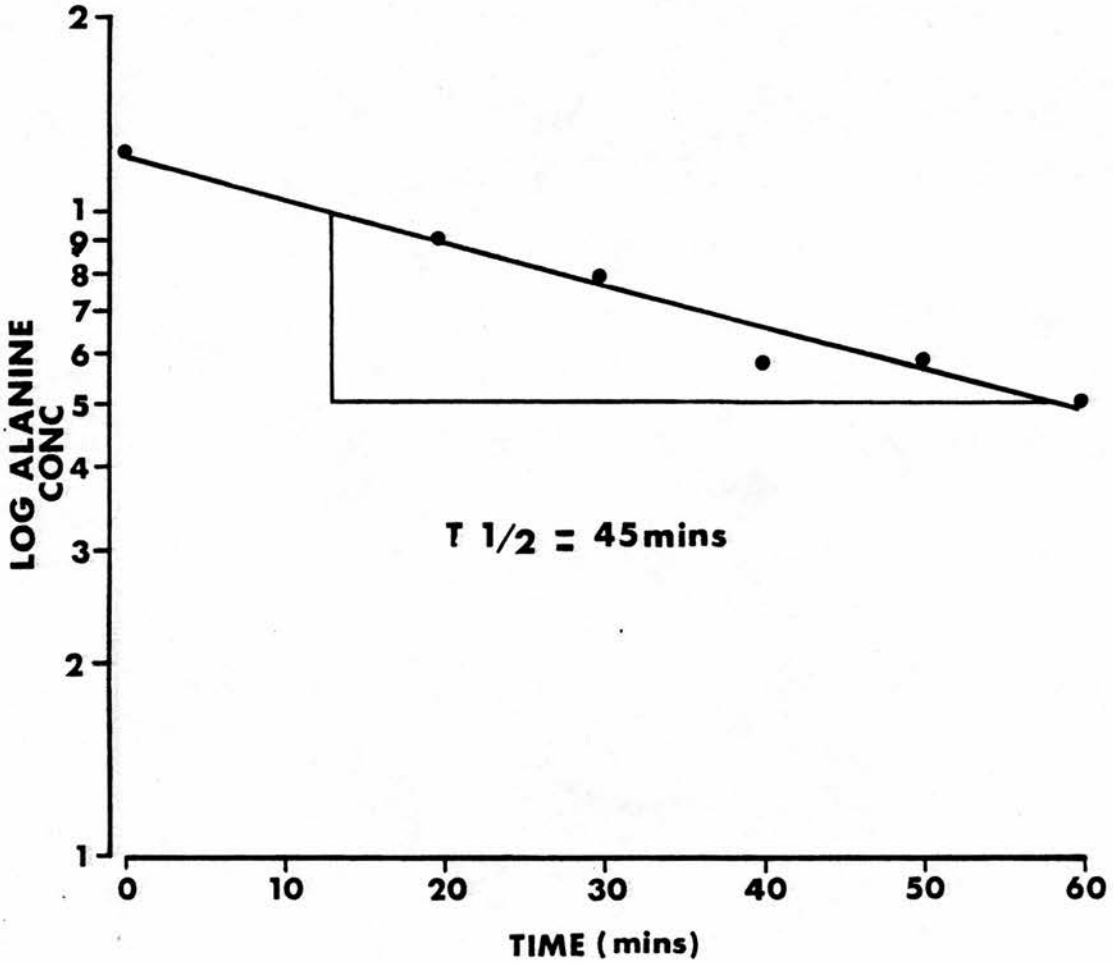


Fig. 11. Graphic measurement of alanine half life

than that in the control group, 73 compared to 47.5 minutes, respectively, there is no significant difference between ranges (TABLE 26).

Once the value for the half-life has been measured a rate constant (k) can be calculated.

$$k = 0.693/t_{0.5} \qquad \text{(units/min)}$$

This represents the logarithm to the base "e" of the ratio of the blood glucose curves divided by the time between samples. Since $t_{0.5}$ is the half life, the ratio of blood glucose levels for that time is by definition 2:1.

$$\log_e 2 = 0.693$$

This can be presented in a simpler form by multiplication by 100. The units of measurement then represent the percentage decrease in blood glucose per minute.

$$k = 0.693 \times 100/t_{0.5} \qquad \text{(\%/min)}$$

Comparing k values for the jaundiced and non-jaundiced groups reveals median values of 0.91 and 1.47 (%/min). There is no significant difference between the ranges of results in the 2 groups (TABLE 26). In addition, a clearance rate which takes into account the glucose distribution volume and individual body weights was devised (Newton et al 1978). The distribution volume is calculated by

extrapolating the slope formed with the logarithmic values of the glucose concentration to the Y axis. This value can be considered the original glucose concentration (G_o). When the quantity of glucose infused (G_i) is divided by G_o the resulting figure represents the distribution volume.

$$\text{DISTRIBUTION VOLUME} = G_i/G_o \quad (1)$$

If this is then divided by the body mass (M) a value for distribution volume per Kg body weight is obtained.

$$\text{DISTRIBUTION VOLUME per Kg BODY WEIGHT} = G_i/G_o \times 1/M \quad (1/Kg)$$

The multiplication of this figure by the rate constant "k", expressed in units per minute, provides a value for the clearance rate relative to the distribution volume per Kg body weight.

$$\text{CLEARANCE RATE} = G_i/G_o \times 1/M \times 0.693/t_{0.5} \times 1000 \quad (ml/min/Kg)$$

The use of this figure should minimise any error brought about by differences in body weight and distribution volume between individuals. When applied to the 2 groups under study here median values of 1.51 and 2.06 (ml/min/Kg) were obtained. There was no significant difference between the ranges of these clearance values.

4.3.2 Preoperative Intravenous Glucose Tolerance Test - FFA Levels.

Fasting FFA levels were significantly different between the groups ranging from 0.26 to 1.52mmol/l (Table 27a) in the jaundiced group and 0.30 to 1.00mmol/l (Table 28a) in the non-jaundiced group. The median values were 0.61 and 0.365mmol/l respectively (Table 29a). The slopes ranged from 0 to -0.013mmol/min (Table 27a) in the jaundiced group and from -0.002 to -0.014mmol/min (Table 28a) in the non-jaundiced group. The median slopes were -0.007 and -0.005mmol/min respectively (Table 29b) and were not significantly different. Graphic representation of these data is displayed in Fig. 12.

4.4.1 Postoperative Alanine Tolerance Test

For reasons already explained only 8 of the patients studied preoperatively were suitable for study postoperatively. Although this means the comparisons of pre and postoperative values limit statistical evaluation, the size of the groups compare well with those in other studies (Elia et al 1980).

As can be seen in Table 30a fasting alanine levels do appear higher in the jaundiced group than in the non-jaundiced group with median fasting levels at 0.19 and 0.14mmols respectively but the differences are not great enough and numerous enough to suggest two separate distributions of results. Median clearance rates of -0.0125 and -0.015mmol/min are statistically similar. When individual patients are considered and the pre and postoperative values are compared, from Tables 6a, 7a and 30a, the following results appear. In the group with biliary obstruction fasting alanine levels almost

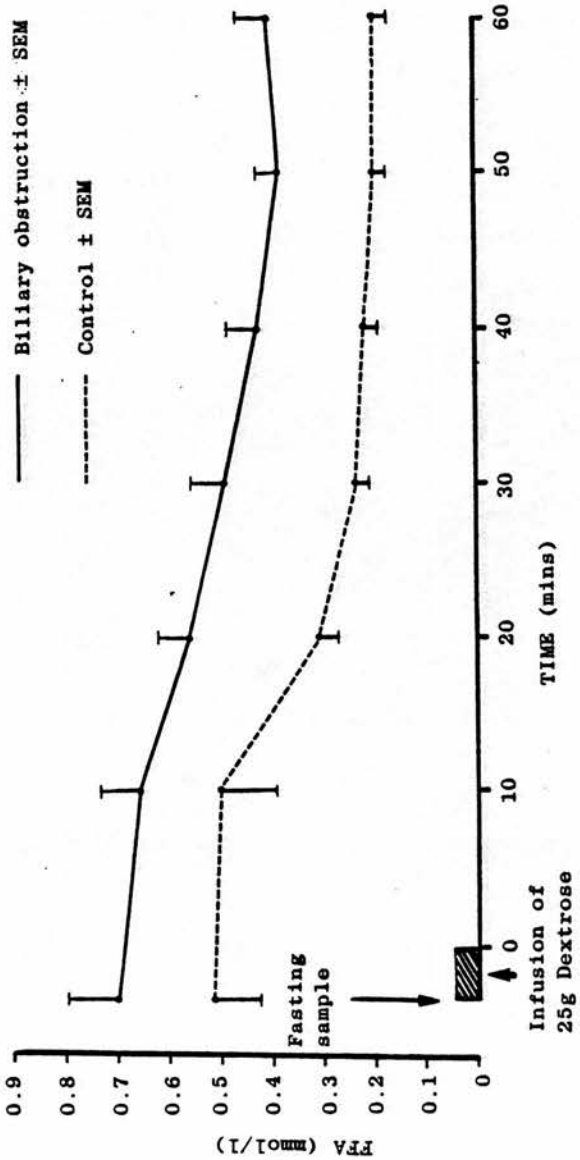


Fig. 12. Preoperative FFA clearance after glucose infusion

doubled in 2 of 4 patients but fell in the remaining 2. The mean change was an increase of 0.055mmol/l. There was also a slight increase in the average clearance rate, for these patients, by 0.004mmol/min although 2 patients showed no change in clearance. The fasting levels in the non-jaundiced group showed a fall postoperatively by an average of 0.06mmol/l. However, preoperative and postoperative results are available on only 2 patients in this group. Clearance rates showed slight increase similar to that found in the jaundiced group with a mean increase of 0.004mmol/min. The changes in clearance rate in both groups is minimal. However, although only a small number of results are available, this suggests that, in the postoperative period, muscle catabolism in the jaundiced group increases while in the non-jaundiced group it decreases.

A comparison of pre and postoperative glucose levels can be made by reference to Tables 9a, 10a and 31a. The mean fasting glucose level in jaundiced patients was lower postoperatively by 0.53mmol/l and was higher in the control group by 0.09mmol/l. Statistical evaluation suggests that it is unlikely that these changes are caused by anything other than chance. There was a negligible change in peak glucose levels in both groups.

No significant changes in FFA metabolism were noted (Tables 12a, 13a, 14a and 32a). On comparing fasting and fasting and peak pyruvate levels in Tables 18a, 19a and 34a no changes were noted between mean pre- and postoperative levels. A comparison of acetoacetate levels was impossible owing to the large number of missing values in Tables 21a, 22a and 35a.

A comparison of mean and median postoperative lactate levels (Table 5) shows that in the group with biliary obstruction there is an increase in the fasting levels after surgery which almost reaches significance when comparing ranges with the Mann Whitney U test. However, in this situation the McNemar test (Seigel 1959) is more appropriate and demonstrates it to be only a trend and not a significant change. This trend is not seen in the control group (Tables 15a, 16a, 33a).

4.5.1 Postoperative Intravenous Glucose Tolerance Test.

As with the postoperative alanine tolerance test the most valuable way to assess the data relating to postoperative glucose clearance is by comparing them with the appropriate individual preoperative levels. For these analysis Tables 24a, 25a, 27a, 28a, 36a and 37a need to be examined.

Fasting glucose levels in the jaundice group were not markedly different before and after surgery. The glucose clearance rates were almost identical on both occasions. Similarly, FFA fasting levels and clearance did not differ significantly before and after surgery.

4.6.1 Nutritional Status Measurements

Nutritional status, in terms of muscle mass, is an important consideration when examining alanine clearance as the ability of the individual to release alanine from muscle is likely to be related to muscle mass. The results of a number of anthropometric and

Table 5

The alanine clearance test
Fasting lactate levels before and after surgery (mmol/l)

	Patient	Preop	Postop
Jaundiced	4	1.08	0.96
	5	0.82	0.64
	8	0.72	1.64
	9	0.27	1.02
	Mean (SD)	0.72 (0.34)	1.07 (0.42)
	Median	0.77	0.99
Non-jaundiced	6	0.56	0.37
	9	0.54	0.96
	10	0.99	0.88
	Mean (SD)	0.70 (0.25)	0.74 (0.32)
	Median	0.56	0.88

biochemical measurements are recorded in Tables 38 and 39. As can be seen from the comparison of individual ranges no difference in the percentage of weight loss, arm muscle circumference, grip strength or lean body mass for the jaundiced and non-jaundiced patients could be found (Fig. 13). Serum albumin, transferrin and thyroxine binding prealbumin were also similar. Total body potassium values were only available on jaundiced patients.

Although there were no significant differences between the groups of patients with regard to anthropometric assessment this did not necessarily imply that an abnormality in one method of assessment could predict the trend in another measure. By comparing normal and abnormal values of each method of assessment with all other methods, very little correlation could be found.

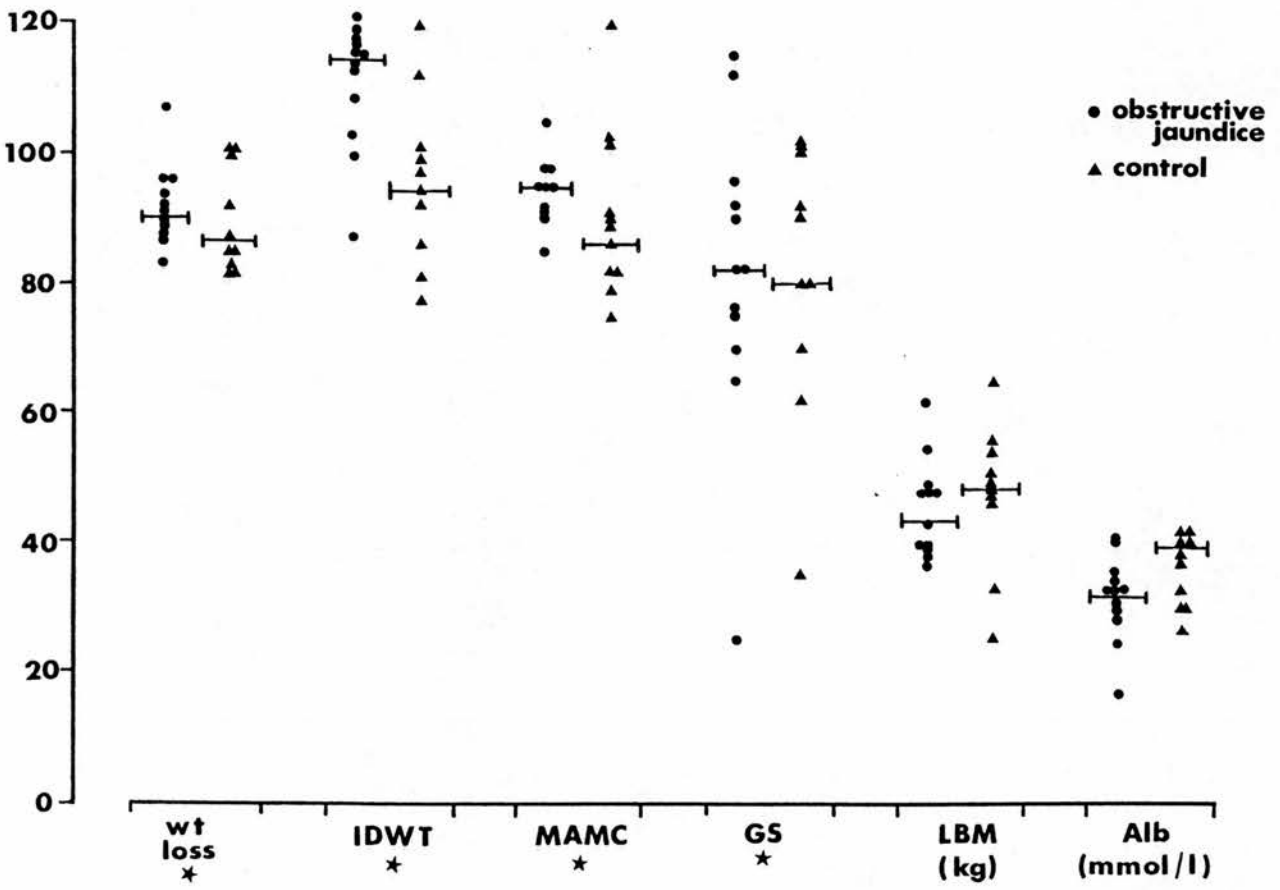
Despite the low numbers having total body potassium estimations a significant correlation, $p = .01$, was found between these values and prealbumin levels. In addition, prealbumin levels correlated well with serum albumin levels ($p < .05$) and arm muscle circumference with estimates of deviation from ideal body weight. However, no other relationships were found.

Renal function, (Table 40), recorded in blood urea and creatinine levels, urinary urea output and creatinine clearance was also similar between study and control groups. Difficulty was encountered in collecting 24 hour urine specimens on some of the control patients.

An examination of serum albumin, alkaline phosphatase and aspartate transaminase in Table 41 revealed significant differences between the jaundiced and non-jaundiced groups in all parameters.

Fig. 13. Comparison of preoperative anthropometric and nutritional data

wt loss = weight loss when compared to reported weight in health, IDWT = ideal weight from actuary tables, MAMC = mid arm muscle circumference, GS = grip strength, LBM = lean body mass (Durnin & Womersley 1974), Alb = serum albumin



DISCUSSION

5.1.1 Discussion

Alanine plays a central role in protein and amino acid metabolism in peripheral muscle, and in hepatic gluconeogenesis. It is quantitatively the major gluconeogenic amino acid (Felig 1973). Following protein degradation in the peripheral muscle, during a period of fasting or stress, it acts as a transport molecule between the periphery and the liver where it is converted to urea and the carbon skeleton necessary for gluconeogenesis. In the fed individual excess circulating amino acids are converted to alanine thus also contributing to this metabolic cycle.

5.2.1 Preoperative alanine clearance study

Since 1973 when Felig first reviewed the importance of alanine in muscle metabolism the alanine clearance study has been employed by several workers in an attempt to assess the rate of muscle catabolism, and its controlling factors, in starved or stressed individuals. In most studies patients received a bolus of L-Alanine following an 8-12 hour fast, and the substrate, metabolite and hormonal concentrations were recorded for 60 minutes following infusion (Royle et al 1977, Elia et al 1980, Royle and Kettlewell 1981, Fernandes and Blom 1974, Genuth and Castro 1974, Nosandini et al 1981, Felig et al 1969b, Swaminathan et al 1981 and Muller et al 1971).

The dose of alanine being offered varied between centres. Some workers used a standard dose of 10 or 12g L-alanine infused over 3-4 minutes (Royle et al 1977, Elia et al 1980, Royle and Kettlewell 1981, Felig et al 1969b, Swaminathan et al 1981) while others offered a

weight related dose (Muller et al 1971, Genuth and Castro 1974, Fernandes and Blom 1974, Nosandini et al 1981). In only one study (Muller et al 1971) dogs were the subject under study, and in another (Genuth and Castro 1974) the alanine was offered orally instead of parenterally. The present study was conducted in a similar manner to that used by Royle et al (1977), Elia et al (1980) and Royle and Kettlewell (1981).

In an alanine clearance study an examination of fasting alanine levels and alanine clearance rates provides information about its production from muscle and its hepatic clearance. In the present study preoperative fasting alanine levels were similar in both jaundiced and non-jaundiced groups. This would imply that either the rates of muscle alanine release and hepatic clearance are similar in both groups or any variations in one of these mechanisms is compensated for by an appropriate change in the complementary process.

When fasting levels in this study are compared to those in other studies it can be seen that they are significantly lower than those found by most other workers. Felig (1969b) recorded fasting alanine levels of 0.25mmol/l, almost twice the median levels found here. Similarly, Royle et al (1977), Elia et al (1980) and Royle and Kettlewell (1981) noted mean fasting levels in excess of 0.22mmol/l, a level exceeded in only 2 non-jaundiced subjects and no subjects with biliary obstruction in the present study. Indeed even in the group of patients with muscle dystrophy, studied by Elia, the mean level was 0.224mmol/l. Swaminathan (1981) recorded fasting alanine levels above 0.5mmol/l in healthy subjects, his lowest mean value, of 0.297mmol/l,

being found in a postoperative group without sepsis.

Since no "normal" controls were included in this study absolute comparisons with other work cannot be made. Nevertheless, the homogeneity of values in the present work and the use of standard solutions to test the analytical methods confirms the validity of the values recorded here.

The implication is, therefore, that in malignant disease, a situation not previously studied with the alanine clearance test, a mechanism seems to prevail which maintains unusually low fasting alanine levels.

Royle and Kettlewell (1981) noted that ketone bodies, acetoacetate and beta-OH-butyrate, exert a controlling influence on alanine released from muscle. Beta-OH-butyrate levels were not recorded in this study but the normal fasting ratio of acetoacetate to beta-OH-butyrate is 1:1 (Swaminathan 1981, Royle and Kettlewell 1981, Elia et al 1980, Nosandini 1981). Comparisons with the results of others reveals that the acetoacetate levels in this study are higher. If, as reported by Royle and Kettlewell (1981), ketone bodies exert a controlling influence on the release of alanine from muscle this may account for the lower fasting alanine levels seen here. Although fasting levels of pyruvate and lactate are similar to these found by others, the glucose levels found here are much lower. This may mean that the individuals under study here are well adapted to starvation, maximally limiting muscle catabolism and alanine derived gluconeogenesis, and using only minimal quantities of glucose for energy. If free fatty acids (FFA) were being preferentially used for energy production then it might be expected that FFA levels would be

higher than normal. In fact, only one previous study of alanine clearance recorded FFA levels (Genuth and Castro 1974) and although they were similar to the levels found here lactate, pyruvate or ketone body levels were not measured, thus making meaningful comparisons difficult.

In this study of patients with biliary obstruction and non-jaundiced control subjects, all of whom were suffering from some form of malignant disease, no difference was noted in fasting alanine or glucose levels or in fasting lactate pyruvate or acetoacetate levels. FFA levels were probably similar but difficulty in measuring them due to the high bilirubin concentration in the serum of jaundiced patients made precise comparisons impossible.

However, no definite conclusions about muscle catabolism based solely on the measurement of fasting levels can be made since a dynamic equilibrium exists between alanine production from muscle and hepatic clearance. Alanine clearance studies demonstrated the relative importance of these two mechanisms.

When compared with the results of others (Elia et al 1980, Royle and Kettlewell 1981, Swaminathan 1981) the median alanine half life is greatly increased in this study. Although the range of values for alanine half-life is very wide in both jaundiced and non-jaundiced groups no significant difference is seen between these groups. Not only is there no significant difference between the alanine clearance rates in jaundiced and non-jaundiced groups, but the clearance rates in this and other studies are also similar. The apparent difference between 2 separate methods for examining disappearance of an infused substrate highlights the difficulty encountered in analysing such

results.

Since the calculation for clearance rate takes into account variations in distribution volume and body weight this may imply that the wide variations found in half life values was as a result of a wide variation in these parameters. An examination of the body weights of individuals studied by Elia et al and Royle and Kettlewell throws doubt on this theory as similar ranges of these parameters were found in the absence of a wide range of alanine half-lives.

However, it can be assumed that alanine clearance, whether measured by clearance rate (ml/min/kg) or half life (mins) is either normal or reduced in comparison to other studies and is unaffected by the presence of biliary obstruction. Since fasting alanine values are low in both groups studied here it can be concluded that these low levels are a result of markedly decreased release of alanine from muscle. This would suggest that patients who are cachectic as a result of malignancy may display a reduced catabolic drive. Such a conclusion is very difficult to accept in view of the frequency with which cachexia is associated with malignancy.

Jeevanandam et al (1984) notes a lower blood alanine concentration in cancer patients than in fasted normal subjects despite a similar or slightly increased alanine flux from the peripheral muscle. He suggests that this is part of an increased catabolic drive to make available increased quantities of glucogenic precursors. This would imply that estimates of the rate of clearance of unphysiological quantities of alanine from the venous circulation is valueless as an index of the clearance rate of endogenously produced alanine. This is a strong argument for the abandoning of

alanine clearance studies except perhaps where tracer techniques are used.

After infusion of alanine, the pyruvate, lactate, glucose and acetoacetate levels varied only slightly and did not appear to deviate significantly from fasting levels. Royle and Kettlewell noted a fall in ketone body levels after alanine infusion in septic patients. Although fasting levels were similar in this study no such change was noted in response to alanine infusion. No explanation for this can be proposed.

Changes reported in glucose concentration by Royle and Kettlewell are tiny and probably not significant. Indeed Fernandes and Blom (1974) suggest that an overnight fast is not long enough to produce a significant glucogenic response from alanine administration.

Only minor variations in pyruvate and lactate concentrations were noted after alanine infusion, none of which reached statistical significance. The only major change recorded was a dramatic reduction in FFA concentrations suggesting a significant change in energy substrate. Since the majority of studies of alanine clearance have ignored alternative energy sources no comparisons can be drawn. The presence of biliary obstruction does not affect the rate of FFA clearance in response to alanine infusion.

In only one report (Elia et al 1980) have patients been studied who have some degree of liver dysfunction. They reported four patients with cirrhosis having increased alanine half lives and decreased clearance rates, thus demonstrating the effect of serious hepatocellular damage. Fasting alanine levels in these patients were

normal, implying a reduced rate of alanine release from muscle, and, suggesting there must be a reduced rate of muscle catabolism in the presence of such reduced clearance. Although the half lives recorded in the present work are longer than those found in the cirrhotic patients above, clearance rates are normal. This suggests that the clearance of alanine in biliary obstruction is more efficient than in the patient with hepatic cirrhosis.

The main aim of this study was to determine the effect of biliary obstruction on gluconeogenesis from alanine and on muscle catabolism. No differences were noted between the jaundiced and non-jaundiced groups in terms of substrate and metabolite levels before and during the alanine clearance test. A relative homeostasis obtains and controlling mechanisms are of secondary importance. After detailed discussion, therefore, it was considered unnecessary to examine insulin, glucagon and growth hormone levels although their importance has been demonstrated in the past (Felig 1973, Royle and Kettlewell 1981).

In the same way that the amount of functioning hepatic tissue can affect the rate of alanine clearance it might be assumed that the available quantity of muscle may affect alanine production.

The state of nutrition was recorded in all patients in this study. Malnutrition varied from a minimal to an advanced degree. Numerous indices of nutritional status were recorded. As has already been mentioned, individual indices of nutritional status are of limited value in determining the degree of malnutrition. This is due to the serious limitations of the measuring techniques or the limited application of the result to the whole individual.

A comparison of techniques to determine whether a predictive correlation existed between them showed little similarity in results, a significant association being found only between total body potassium, serum albumin and thyroxine binding prealbumin, and between arm muscle circumference and weight loss related to ideal weight, and forearm muscle circumference and real weight loss. This lack of similarity between results confirms the poor predictive value of any single measurement of nutritional status. When ranges of such measurements are examined alongside the respective fasting alanine concentration, no association can be found between nutritional status and such levels. This also holds true for alanine clearance rates and half lives.

Therefore, apart from the need to record body weight for the calculation of alanine clearance, no additional value exists in recording complex parameters of nutritional status. The lack of association between alanine metabolism and nutritional status throws doubt on the importance of the equilibrium of the glucose alanine cycle in muscle catabolism. It may be, as suggested above, that at the time of study the active phase of catabolism may have passed, especially in chronic illness such as malignancy.

The study of preoperative fasting levels and clearance rates in patients with malignancy demonstrates no abnormality attributable to biliary obstruction. When compared to other studies the fasting levels found here are very low despite clearance rates being similar and alanine half lives being prolonged. This suggests that a controlling mechanism appears to exist which may be peculiar to the patient with malignancy. Acetoacetate levels are high and this may

reflect an overall increase in ketone body production which is known to limit alanine release. Although the anorexia of malignancy may contribute to this no association could be drawn between nutritional status and either fasting alanine levels or alanine clearance.

The present study has suffered from having no group comprising healthy controls but its aim was chiefly to determine the effect of biliary obstruction on alanine metabolism. Since alanine homeostasis did not differ between the study and control groups, measuring the levels of insulin, glucagon and growth hormone would provide results which would be of little import.

In conclusion, therefore, inasmuch as it can be determined from this work, it would appear that when considering preoperative nutritional support for the patient with biliary obstruction no special formulations are required. Preoperative nutritional support may improve postoperative prognosis in biliary obstruction, but no specific abnormalities in the glucose alanine cycle exist to support this view. It is likely that studies of alanine clearance are either unable to demonstrate the relevant metabolic changes or are unassociated with nutritional metabolism in otherwise uncomplicated biliary obstruction.

5.2.2 A comparison of pre and postoperative alanine clearance.

Of the 20 patients who were studied preoperatively, 4 with biliary obstruction and 3 with normal liver function were also studied postoperatively. A comparison of preoperative and postoperative data shows an apparent 50 percent increase in the rate of alanine clearance

in the patients with biliary obstruction which is not seen in the control subjects.

Postoperative alanine clearance studies have been carried out by 3 groups of workers (Royle and Kettlewell 1981, Swaminathan et al 1981 and Elia et al 1980) but in only one study (Elia et al 1980) were the patients studied both pre and postoperatively. Their results indicate that the fasting alanine level fell within 6 hours of surgery being lowest on the second postoperative day. After that it was shown to return towards normal by the end of the first postoperative week. The clearance rate was maximal and half life minimal by the third postoperative day and these too had begun to return to normal by the 7th postoperative day.

In the present study it is considered that such small cohorts cannot provide conclusive or reliable data despite the use of such numbers in other studies (Elia et al 1980). Discussion of these data is therefore limited.

The non-jaundiced group demonstrated a drop in the median fasting alanine level which is not statistically significant, and the median half life and median alanine clearance rate did not change. However, in the group with biliary obstruction there is a significant fall in the half life and rise in the clearance rate of around 50 percent. The median fasting levels did not change.

It has already been noted that nutritional status measurements do not seem to correlate with alanine metabolism and for the most part no significant change is recorded in the postoperative groups under study. However, the midarm muscle circumference, showed a significant

reduction postoperatively. This change is only significant in the group with biliary obstruction.

Although this change reaches significance in only one parameter there is a general trend in other parameters towards a more malnourished state. When this is considered in conjunction with an increased alanine clearance rate, in the group with biliary obstruction, an increased rate of muscle catabolism seems likely. This, therefore, offers further evidence in favour of an aggressive nutritional support program for the patient recovering from surgery for biliary obstruction.

5.3.1 The Intravenous Glucose Tolerance Test

Glucose clearance has been studied extensively in many physiological and pathological situations. Three major pathological situations were under study here: patients with malignant disease; patients with malignant obstructive jaundice; and, the same patients following surgery. Since the first report by Freund (1885) many authors have remarked on the frequency of glucose intolerance in malignancy (Friedwald and Grove 1920, Glicksman and Rawson 1956, Weisenfeld et al 1962, Benjamin and Romney 1964, Marks and Bishop 1957, Jasani et al 1978 and Schein et al 1979).

Although a reduced rate of glucose clearance was noted in some of the patients under study here, both reduced and increased rates have been reported in the past in relation to a variety of malignant processes. Hypoglycaemia has been a reported symptom of leiomyosarcoma and of hepatocellular carcinoma (Chandalia and Boshell

1972, Kreisberg and Pennington 1970) and in 1966 Unger reviewed 98 similar cases reported in the literature to that date. However, impaired glucose tolerance is a much commoner problem, several cases of which were found in the present study. No case of hypoglycaemia was encountered.

No patient had a fasting glucose level above normal, but in 11 (55%) the blood glucose failed to return to normal within an hour. This occurred equally in both groups. Therefore, in the patient with malignant disease, a fasting blood glucose may mislead the observer into believing that normal glucose homeostasis exists.

In 1920, Friedenwald and Grove noted 31 of 32 previously reported cases of gastrointestinal malignancy with high fasting glucose levels and impaired tolerance to an oral glucose load. Glicksman and Rawson (1956) found impaired glucose tolerance in 37% of 628 patients with all types of cancer, and this was also found in 62% of 31 patients with cancer reported by Weisenfeld et al (1962), and in 56% of 75 patients with endometrial cancer studied by Benjamin and Romney (1964).

Decreased glucose tolerance may reflect either diminished secretion of, or, a diminished response to endogenous insulin. Schein et al (1979) suggests that a metabolic toxin is released from the tumour which reduces insulin sensitivity in the individual. However, Jasani et al (1978) recorded an impaired insulin release, in patients with malignancy, and noted an association between the severity of glucose intolerance and the degree of cachexia. The mean clearance constant (k) was 1.06 (SD=0.27) for the cachectic patients, 1.63 (SD=0.23) for the non-cachectic patients with cancer, and 1.64

(SD=0.34) for the normal subjects. In the present study k values ranged from 0.15 to 2.24 and 45% (9/20) were within the diabetic range. No association was found between any measure of nutritional status and the glucose clearance rates.

Glucose intolerance is also recognised in obstructive jaundice. Solar et al (1974) examined 23 patients with obstructive jaundice by means of an oral glucose tolerance test. Of these patients, 12 had carcinoma of pancreas, 6 gall stone disease, 4 had drug induced cholestasis and one had hepatitis. Seven of those with carcinoma and 6 of the remainder were shown to have a diabetic response to a glucose challenge. These data implicate the obstruction rather than the absolute pathology as the causative factors in glucose intolerance in obstructive jaundice in contrast to the present findings.

According to the criteria of Lundbaek (1962) it is suggested that the patients with a glucose clearance constant (k) less than 0.9 (%/min) can be considered diabetic, and those with a k value greater than 1.05 are normal. Patients with a k value between 0.9 and 1.05 can be considered mildly diabetic.

In the present study, of the group with obstructive jaundice 6 (55%) fell into the diabetic group, 3 (27%) into the non-diabetic group and 2 (18%) could be considered mildly diabetic. In the control group, 3 (38%) were diabetic and the remainder, 5 (62%), were non-diabetic. There is no significant difference between these groups despite a trend to poorer glucose tolerance in the jaundiced group. Despite the small numbers in each group it can be assumed that the presence of obstructive jaundice may not be the sole contributing factor to the impaired glucose tolerance in this condition, and that

malignancy must be considered to be an additional factor.

It must also be borne in mind that the results of Solar et al (1974) were based upon an oral glucose tolerance test. The present study avoids the influence of altered gastrointestinal absorption commonly found in obstructive jaundice as well as the complex endocrine response to intraluminal glucose.

Following partial or complete resolution of jaundice, further study showed no improvement in glucose clearance. The presence of biliary obstruction, therefore, did not appear to alter the rate of glucose clearance. In both groups of patients under study, the glucose clearance rate was impaired to a similar extent and the presence of biliary obstruction produced no noticeable deterioration in this rate. The earlier studies of alanine metabolism can, therefore, be considered in isolation from variations in glucose clearance.

SUMMARY

5.4.1 SUMMARY

Hepatic gluconeogenesis from alanine and the intravenous clearance of glucose have been studied in patients with malignant disease, some of whom had biliary obstruction. A comparison of fasting substrate and metabolite levels, and, substrate clearance rates were compared in jaundiced and non-jaundiced groups, before and after surgery. Postoperative studies were conducted after biliary obstruction had been relieved. This allowed the comparison of paired data where the only differences were the presence and absence of obstructive jaundice before and after surgery, respectively.

After an overnight fast, basal alanine levels, alanine clearance rates and the associated substrate metabolism were similar in both study and control groups. In the subgroups studied both pre- and postoperatively no change was noted in the control group, but clearance rates in the previously jaundiced group increased significantly by 50 percent. Since this change occurred in the presence of unchanged fasting alanine levels this implies there is an increase in the production of muscle-derived alanine. Such a change is not normally seen in the late postoperative period. In the absence of adequate nutritional support this could lead to an increased rate of protein catabolism. Aggressive and prolonged nutritional support is recommended after surgery for biliary obstruction.

Fasting lactate levels also increased in the postoperative group recovering from biliary obstruction. The reason for this is uncertain but may indicate a change to an alternative fuel substrate.

The clearance of an intravenous glucose load was prolonged in

both groups of patients. In the control group this was, presumably, as a result of the existing malignant disease. Whereas this may also be true in the group with biliary obstruction, abnormal hepatic function was examined as a contributory factor. The postoperative glucose clearance study demonstrated no change in clearannce rate suggesting that the presence of biliary obstruction causes no further deterioration in glucose tolerance in the patient with malignant disease. These results also demonstrate that no serious alteration to glucose clearance existed in the patients in whom alanine tolerance tests were carried out.

FFA levels were recorded to determine the degree of conversion to FFA as an alternative to carbohydrate as a fuel source. In both the alanine and glucose clearance tests there was evidence of a conversion to FFA for energy production in the fasting patient. FFA levels fell following infusion of both substrates.

It might be assumed that fasting alanine levels and alanine production might be affected by nutritional status and estimations of muscle bulk. However, no association was found between nutritional status and either glucose or alanine metabolism.

5.4.2 Further work

This work represents a small inroad into nutritionally related metabolic studies in the patient with biliary obstruction. Its weaknesses lie in the inability to follow, except by inference, the fate of the various components of the alanine molecule. If further work of this kind is considered it should employ labelling techniques

to obtain maximal information. In addition, the study of steady state environments brought about by constant infusion techniques, would make interpretation of results easier.

The present study has failed to indicate preoperative abnormalities in patients with biliary obstruction which might account for the postoperative morbidity and mortality rates seen in this condition. Other metabolic parameters should also be investigated. A study of the rate of clearance of lipid emulsions, would be valuable, and has been planned. By labelling a trioleate molecule with C^{14} the rate of $C^{14}O_2$ production and the decrease of C^{14}-labelled trioleate would accurately record the drive to lipid metabolism in these patients.

Aggressive postoperative nutritional support for a prolonged period should also be considered in patients having surgery for biliary obstruction. However, even in centres where such patients are regularly seen the population size of a study and a control cohort would need to be so large as to make the study last for an unacceptably long period.

ACKNOWLEDGEMENTS

This work was entirely conducted in the Royal Postgraduate Medical School and Hammersmith Hospital, London. I am grateful to the University of London for allowing me to complete this study.

I am indebted to Professor L H Blumgart for appointing me to the post of Research Fellow in his department and for offering me continual encouragement and consideration. I am also grateful to Mr I S Benjamin and Mr G A D MacPherson for their help, cooperation and enthusiasm during the course of this work.

I am very grateful to Dr G M Hall, Miss J Paterson and Miss S M Sapsed, Department of Anaesthetics, Royal Postgraduate Medical School for their helpful discussion at each stage of the study and for their detailed assistance with laboratory procedures.

I am deeply grateful to Geistlich Sons, Chester for funding this work, and particularly to Mr D Cottier, the Director of Medical Research of the Company for his unbiased generosity and support.

During the final stages of printing and editing this thesis Dr DBA Silk, Consultant Physician, Central Middlesex Hospital, Acton Lane, London NW10, offered invaluable assistance and criticism.

Finally, I would like to thank my wife for her encouragement, support and typing skills throughout the preparation of this manuscript.

APPENDIX 1

Preoperative alanine tolerance test

TABLES 6a. Alanine levels (mmol/l) in jaundiced patients

		1	2	3	4	5	6	7	8	9	10	
	0	0.19	0.09	0.16	0.14	0.13	0.16	0.22	0.08	0.07	0.14	
	10	1.64	1.33	1.83	1.73	1.49	1.74	1.24	1.30	1.62	1.44	
	20	1.28	1.22	1.61	1.18	1.41	1.33	1.21	0.67	1.13	1.13	
TIME	30	0.99	1.05	1.43	0.99	1.03	1.26	0.94	0.77	1.24	0.87	
(mins)	40	1.08	0.95	1.37	0.93	0.93	0.95	0.80	0.72	1.11	0.89	
	50	0.84	0.87	1.37	0.80	0.96	0.73	0.50	0.61	1.05	0.83	
	60	0.53	0.80	0.94	0.75	0.90	0.66	0.51	0.59	0.64	0.60	
Slope		-.017	-.010	-.014	-.011	-.011	-.019	-.018	-.003	-.012	-.011	(mmol/min)
Half life		34.0	82.5	60.0	60.0	78.5	36.0	28.5	145.0	52.0	51.0	(mins)
k		2.04	0.84	1.16	1.16	0.88	1.93	2.43	0.48	1.33	1.36	(%/min)
Y-intercept		1.99	1.34	2.08	1.45	1.52	2.07	1.95	0.81	1.7	1.43	(mmol/l)
Clearance rate		23.61	13.15	10.54	13.94	9.85	19.92	28.85	11.58	15.8	20.32	(ml/min/Kg)

TABLE 6b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	37.10	
Degrees of	V1	1	$p < .05$
Freedom	V2	45	
Linearity coefficient	F2	0.14	
Degrees of	V1	3	N.S.
Freedom	V2	45	

Preoperative alanine tolerance test

TABLE 7a. Alanine levels (mmol/l) in non-jaundiced patients

		1	2	3	4	5	6	7	8	9	10	
	0	0.16	0.20	0.11	0.29	0.13	0.13	0.11	0.18	-	0.22	
	10	1.19	1.92	1.86	1.79	1.45	1.92	1.91	1.52	1.27	1.45	
	20	1.25	1.73	1.45	1.46	1.28	0.77	1.54	1.08	0.92	1.10	
TIME	30	0.75	1.39	1.11	1.21	0.96	0.97	1.49	0.94	0.79	1.13	
(mins)	40	-	1.05	1.01	1.39	0.61	0.77	1.34	0.94	0.58	1.08	
	50	0.64	0.44	1.09	1.15	0.68	1.04	1.39	0.74	0.58	0.68	
	60	0.66	1.09	0.88	1.07	0.59	0.98	1.33	0.77	0.50	0.60	
Slope		-.013	-.022	-.012	-.008	-.017	-.005	-.005	-.008	-.011	-.015	(mmol/min)
Half life		31.0	29.5	67.5	84.0	38.5	>500	160	75.0	45.0	46.5	(mins)
k		2.24	2.35	1.03	0.83	1.8	0	0.43	0.92	1.54	1.71	(%/min)
Y-intercept		1.95	2.66	1.64	1.63	1.69	0.76	1.63	1.28	1.21	1.77	(mmol/l)
Clearance rate		25.42	20.54	17.91	10.95	20.59	0	8.9	11.53	20.4	20.22	(ml/min/Kg)

TABLE 7b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	19.60	
Degrees of	V1	1	$p < .05$
Freedom	V2	61	
Linearity coefficient	F2	0.35	
Degrees of	V1	5	N.S.
Freedom	V2	61	

TABLE 8a. Summary of preoperative alanine clearance

	Biliary Obstruction (Median)	Control (Median)	
Fasting levels (mmol/l)	0.135 (0.149)	0.168 (0.184)	NS
Slope (mmol/l)	-0.011 (0.017)	-0.01 (0.018)	NS
k (%/min)	1.22 (1.97)	1.28 (2.35)	NS
Half life (min)	56 (116.5)	56.25 (> 130)	NS
Clearance (ml/min/Kg)	14.87 (19)	18.03 (25.42)	NS

Figures in this table represent median values of ranges. The figures within brackets denote the extent of each range. Ranges were compared using the Mann Whitney U test. NS denotes no significance ($p > 0.1$).

Preoperative alanine tolerance test

TABLE 9a. Glucose levels (mmol/l) in patients with obstructive jaundice

		1	2	3	4	5	6	7	8	9	10
	0	2.97	3.66	3.76	5.44	5.33	3.50	3.33	4.41	3.50	3.93
	10	3.55	4.56	4.18	5.30	4.92	3.61	3.38	5.03	3.42	3.38
	20	4.00	4.33	4.32	5.39	6.17	3.71	3.63	5.22	4.01	3.68
TIME	30	4.19	4.58	4.27	5.21	5.56	3.38	3.65	5.00	3.94	3.31
(mins)	40	4.01	4.27	-	5.69	4.02	3.18	3.85	5.24	3.76	3.80
	50	3.86	4.09	4.39	5.15	3.79	3.66	3.65	5.38	2.41	4.24
	60	3.12	4.09	4.68	5.71	3.87	2.86	3.75	4.39	3.67	3.77
SLOPE (mmol/min)		.004	.001	-.004	.003	-.031	-.008	.007	.002	-.006	.005

TABLE 9b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	0.074	
Degrees of	V1	1	N.S.
Freedom	V2	62	
Linearity coefficient	F2	0.524	
Degrees of	V1	5	N.S.
Freedom	V2	62	

Preoperative alanine tolerance test

TABLE 10a. Glucose levels (mmol/l) in non-jaundiced patients

		1	2	3	4	5	6	7	8	9	10
	0	4.37	5.50	4.79	3.01	3.75	3.22	3.62	5.33	3.59	2.75
	10	6.09	4.48	3.34	3.63	4.01	3.98	3.59	5.09	3.98	2.83
	20	5.48	5.20	3.80	4.01	3.97	3.24	4.67	4.96	3.92	3.02
TIME	30	5.00	5.39	3.89	3.87	4.03	4.18	5.14	5.25	4.30	3.14
(mins)	40	-	5.52	3.36	4.05	4.04	3.84	4.48	5.02	4.22	3.18
	50	6.51	5.50	4.18	3.81	4.09	3.96	4.41	4.22	4.69	3.10
	60	4.69	5.97	4.20	3.89	4.08	3.01	4.91	4.72	4.46	3.35
SLOPE (mmol/min)		.006	.013	-.002	.011	.004	0	.019	-.013	.015	.009

TABLE 10b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	1.338	
Degrees of	V1	1	N.S.
Freedom	V2	62	
Linearity coefficient	F2	0.178	
Degrees of	V1	5	N.S.
Freedom	V2	62	

TABLE 11a. Preoperative Alanine Tolerance Test

Summary of fasting glucose levels

	MEDIAN (mmol/1)	RANGE (mmol/1)	N	
Bilirubin > 100umol/1	3.635	2.75	10	NS*
Bilirubin < 100umol/1	3.71	2.47	10	

TABLE 11b. Preoperative Alanine Tolerance Test

Summary of peak glucose levels

	MEDIAN (mmol/1)	RANGE (mmol/1)	N	
Bilirubin > 100umol/1	4.71	3.16	10	NS*
Bilirubin < 100umol/1	4.265	2.46	10	

* Mann Whitney U test.

Preoperative alanine tolerance test

TABLE 12a
Free fatty acid levels (mmol/l) in patients with obstructive jaundice

		1	2	3	4	5	6	7	8	9	10
	0	0.46	0.89	0.79	1.22	0.91	0.73	0.73	1.32	1.00	0.77
	10	0.37	1.09	0.76	1.32	0.83	0.68	0.46	1.13	0.92	0.67
	20	0.31	1.08	0.71	1.01	0.53	0.77	0.35	0.93	0.80	0.48
TIME	30	0.17	0.84	0.68	0.86	0.61	0.66	0.31	0.87	0.67	0.52
(mins)	40	0.18	0.83	0.55	0.84	0.46	0.49	0.27	0.83	0.58	0.49
	50	0.17	0.74	0.64	0.86	0.50	0.40	0.23	0.76	0.66	0.40
	60	0.24	0.56	0.63	0.69	0.48	0.32	0.32	0.60	0.59	0.33
SLOPE (mmol/min)		-.004	-.007	-.003	-.010	-.007	-.007	-.006	-.011	-.007	-.007

TABLE 12b Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	22.755	
Degrees of	V1	1	$p < .05$
Freedom	V2	63	
Linearity coefficient	F2	0.191	
Degrees of	V1	5	N.S.
Freedom	V2	63	

Preoperative alanine tolerance test

TABLE 13a
Free fatty acid levels (mmol/l) in non-jaundiced patients

		1	2	3	4	5	6	7	8	9	10
TIME (mins)	0	0.41	0.44	0.79	0.66	0.50	0.43	0.68	0.97	0.61	0.59
	10	0.16	0.42	0.38	0.56	0.38	0.31	0.68	1.03	0.60	0.66
	20	0.16	0.39	0.29	0.52	0.34	0.23	0.58	0.95	0.61	0.51
	30	0.11	0.39	0.23	0.36	0.19	0.21	0.50	0.93	0.40	0.63
	40	-	0.36	0.28	0.30	0.15	0.20	0.34	0.68	0.57	0.49
	50	0.09	0.31	0.20	0.31	0.13	0.20	0.28	0.53	0.62	0.48
	60	0.15	0.29	0.22	0.33	0.15	0.17	0.25	0.53	0.61	0.51
SLOPE (mmol/min)		-.003	-.003	-.007	-.006	-.006	-.004	-.008	-.009	0	-.002

TABLE 13b Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	15.307	
Degrees of Freedom	V1	1	$p < .05$
	V2	62	
Linearity coefficient	F2	0.211	
Degrees of Freedom	V1	5	N.S.
	V2	62	

TABLE 14a. Preoperative Alanine Tolerance Test

Summary of fasting free fatty acid levels

	MEDIAN (mmol/l)	RANGE (mmol/l)	N	
Bilirubin > 100umol/l	0.8	0.86	10	$p<.01$*
Bilirubin < 100umol/l	0.595	0.56	10	

TABLE 14b. Preoperative Alanine Tolerance Test

Summary of free fatty acid clearance rates

	MEDIAN (mmol/l)	RANGE (mmol/l)	N	
Bilirubin > 100umol/l	-0.007	0.008	10	NS*
Bilirubin < 100umol/l	-0.007	0.008	10	

* Mann Whitney U test

Preoperative alanine tolerance test

TABLE 15a. Lactate levels (mmol/l) in patients with obstructive jaundice

		1	2	3	4	5	6	7	8	9	10
	0	2.31	1.40	0.68	1.08	0.82	0.92	0.33	0.72	0.27	1.42
	10	2.30	1.01	0.72	0.94	0.89	0.96	0.84	-	0.42	1.59
	20	2.13	1.16	0.58	1.44	0.86	0.76	0.98	1.20	0.40	1.62
TIME	30	1.60	0.89	1.37	1.07	1.15	0.95	0.89	-	0.58	1.61
(mins)	40	2.22	0.80	0.97	1.17	0.80	0.85	0.60	1.50	0.33	1.22
	50	2.31	0.95	0.98	0.81	0.79	0.63	0.87	1.27	0.45	1.28
	60	2.02	0.87	1.10	1.18	0.65	0.94	0.84	1.08	0.45	1.20
SLOPE (mmol/min)		-.003	-.007	.008	0	-.003	-.002	-.004	-.007	-.002	-.006

TABLE 15b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	0.001	
Degrees of	V1	1	N.S.
Freedom	V2	61	
Linearity coefficient	F2	0.097	
Degrees of	V1	5	N.S.
Freedom	V2	61	

Preoperative alanine tolerance test

TABLE 16a. Lactate levels (mmol/l) in non-jaundiced patients

		1	2	3	4	5	6	7	8	9	10
	0	0.35	-	1.38	0.79	0.79	0.56	0.82	1.01	0.54	0.99
	10	0.47	2.41	1.33	0.73	0.97	0.84	0.74	0.91	0.48	1.03
	20	0.49	2.54	1.24	1.00	0.96	0.86	0.34	0.97	0.57	0.84
TIME	30	0.45	2.67	1.45	0.69	1.47	1.00	0.87	1.18	0.78	0.76
(mins)	40	-	2.01	1.40	0.86	1.08	0.80	1.00	.130	2.36	0.73
	50	0.40	2.21	1.21	1.04	0.96	1.01	0.87	1.16	1.06	0.62
	60	0.52	1.66	0.76	0.84	0.94	1.61	1.13	0.93	0.88	0.53
SLOPE (mmol/min)		.001	-.015	-.007	.002	.002	.012	.007	.002	.014	-.008

TABLE 16b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	1.02	
Degrees of	V1	1	N.S.
Freedom	V2	61	
Linearity coefficient	F2	0.691	
Degrees of	V1	5	N.S.
Freedom	V2	61	

TABLE 17a. Preoperative Alanine Tolerance Test

Summary of fasting lactate levels

	MEDIAN (mmol/1)	RANGE (mmol/1)	N	
Bilirubin > 100umol/1	0.83	2.04	10	NS*
Bilirubin < 100umol/1	0.795	1.03	10	

TABLE 17b. Preoperative Alanine Tolerance Test

Summary of peak lactate levels

	MEDIAN (mmol/1)	RANGE (mmol/1)	N	
Bilirubin > 100umol/1	1.38	1.73	10	NS*
Bilirubin < 100umol/1	1.185	2.15	10	

* Mann Whitney U test

TABLE 18a. Preoperative alanine tolerance test
Pyruvate levels (mmol/l) in jaundiced patients

		1	2	3	4	5	6	7	8	9	10
TIME (mins)	0	0.11	0.11	0.12	0.03	0.14	0.06	0.13	0 08	0.03	0.07
	10	0.10	0.12	0.12	0.12	0.15	0.08	0.16	0.16	0.03	0.07
	20	0.16	0.15	0.12	0.12	0.15	0.09	0.14	0.18	-	0.12
	30	0.13	0.16	0.13	0.12	0.18	0.11	0.16	0.14	0.04	0.04
	40	0.12	0.15	0.16	0.09	0.12	0.08	0.13	0.17	0.04	0.07
	50	0.26	0.12	0.14	0.10	0.13	0.08	0.16	0.15	0.02	0.07
	60	0.20	0.11	0.19	0.09	0.15	0.05	0.16	0.08	0.03	0.07
SLOPE (mmol/min)		.002	0	.001	0	0	0	0	0	0	0

TABLE 18b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	1.056	
Degrees of Freedom	V1	1	N.S.
	V2	62	
Linearity coefficient	F2	0.928	
Degrees of Freedom	V1	5	N.S.
	V2	62	

TABLE 19a. Pyruvate levels (mmol/l) in non-jaundiced patients

		1	2	3	4	5	6	7	8	9	10
	0	0.12	0.11	0.10	0.16	0.09	0.06	0.07	0.13	0.08	0.03
	10	0.11	0.15	0.12	0.16	0.14	0.07	0.12	0.13	0.10	0.05
	20	0.13	0.17	0.18	0.17	0.16	0.07	0 09	0.16	0.12	0.05
TIME	30	0.14	0.18	0.10	0.18	0.14	0.10	0.16	0.15	0.13	0.03
(mins)	40	-	0.17	0.10	0.21	0.16	0.08	0.17	0.18	0.13	0.04
	50	0.14	0.17	0.14	0.15	0.14	0.09	0.11	0.16	0.110	0.04
	60	0.13	0.21	0.13	0.19	0.15	0.08	0.10	0.15	0.08	0.04
SLOPE (mmol/min)		0	.001	0	0	0	0	0	0	0	0

TABLE 19b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	2.67	
Degrees of	V1	1	N.S.
Freedom	V2	62	
Linearity coefficient	F2	0.68	
Degrees of	V1	5	N.S.
Freedom	V2	62	

TABLE 20a. Preoperative Alanine Tolerance Test

Summary of fasting pyruvate levels

	MEDIAN (mmol/1)	RANGE (mmol/1)	N	
Bilirubin > 100umol/1	0.019	0.302	10	NS
Bilirubin < 100umol/1	0.094	0.125	10	

TABLE 20b. Preoperative Alanine Tolerance Test

Summary of peak pyruvate levels

	MEDIAN (mmol/1)	RANGE (mmol/1)	N	
Bilirubin > 100umol/1	0.159	0.216	10	NS
Bilirubin < 100umol/1	0.142	0.158	10	

Mann Whitney U test

Preoperative alanine tolerance test

TABLE 21a.

Aceto-acetate levels (mmol/1) in patients with obstructive jaundice

		1	2	3	4	5	6	7	8	9	10
	0	0.05	0.09	0.14	0.06	-	0.09	-	0.09	-	0.14
	10	0.03	0.10	0.10	0.04	-	0.16	-	-	-	0.10
	20	0.02	0.13	0.11	0.07	-	0.15	-	0.06	-	0.10
TIME	30	0.02	0.09	0.10	0.05	-	0.15	-	0.06	-	0.05
(mins)	40	0	0.09	0.10	0.07	-	0.19	-	0.05	-	0.08
	50	0	0.14	0.10	0.05	-	0.16	-	0.05	-	0.11
	60	0.14	0.08	0.06	0.06	-	0.15	-	0.07	-	-
SLOPE (mmol/min)		.001	0	-.001	0	-	.001	-	0	-	-.001

TABLE 21b. Least squares regression analysis for linearity

Regression coefficient	F1	0.05	
Degrees of Freedom	V1	1	N.S.
	V2	40	
Linearity coefficient	F2	0.15	
Degrees of Freedom	V1	5	N.S.
	V2	40	

Preoperative alanine tolerance test

TABLE 22a.

Aceto-acetate levels (mmol/l) in non-jaundiced patients

		1	2	3	4	5	6	7	8	9	10
	0	0.10	0.05	0.06	-	0.08	0.11	0.05	0.09	-	0.09
	10	0.11	0.06	0.04	-	0.05	0.15	0.06	-	-	0.10
	20	0.11	0.05	0.02	-	0.05	0.11	0.11	0.06	0.12	0.07
TIME	30	0.12	0.07	0.04	-	0.01	0.25	0.12	0.06	0.05	0.09
(mins)	40	-	0.06	0.03	-	0.08	0.17	0.14	0.07	-	0.07
	50	0.13	0.06	0.04	-	0.09	-	0.08	0.06	0.08	0.07
	60	0.11	0.04	0.09	-	0.09	0.12	0.07	0.08	0.07	0.08
SLOPE (mmol/min)		0	0	0	-	0	0	.001	0	-.001	0

TABLE 22b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	0	
Degrees of	V1	1	N.S.
Freedom	V2	50	
Linearity coefficient	F2	0.14	
Degrees of	V1	5	N.S.
Freedom	V2	50	

TABLE 23a. Preoperative Alanine Tolerance Test

Summary of fasting aceto-acetate levels

	MEDIAN (mmol/l)	RANGE (mmol/l)	N	
Bilirubin > 100umol/l	0.090	0.092	7	NS*
Bilirubin < 100umol/l	0.087	0.061	8	

TABLE 23b. Preoperative Alanine Tolerance Test

Summary of peak aceto-acetate levels

	MEDIAN (mmol/l)	RANGE (mmol/l)	N	
Bilirubin > 100umol/l	0.014	0.117	7	NS*
Bilirubin < 100umol/l	0.101	0.194	9	

* Mann Whitney U test

Preoperative glucose tolerance test

TABLE 24a. Glucose levels (mmol/l) in patients with obstructive jaundice

		1	2	3	4	5	6	7	8	10	11	12
	0	3.12	4.02	5.85	5.00	4.08	2.83	5.49	5.73	3.68	3.73	3.93
	10	16.00	11.23	10.79	10.61	9.19	9.71	14.15	13.16	9.50	12.67	10.48
	20	9.43	10.47	9.73	9.75	7.80	8.68	12.41	10.41	9.99	10.35	8.72
TIME	30	8.45	10.01	8.43	8.89	7.16	7.84	12.00	10.37	8.77	10.49	7.32
(mins)	40	7.05	8.80	7.17	8.84	6.29	6.33	10.07	9.52	8.25	8.71	6.55
	50	7.23	7.87	7.03	6.87	5.50	5.37	10.09	9.73	7.05	7.88	5.96
	60	7.02	7.48	6.81	6.57	5.77	5.62	10.00	9.20	6.73	6.68	5.90
SLOPE (mmol/min)		-.060	-.081	-.072	-.084	-.057	-.086	-.059	-.031	-.082	-.100	-.070
Y-intercept (mmol/l)		11.7	12.4	11.5	11.9	9.2	10.8	14.0	10.1	12.0	13.0	10.5
Half life (min)		63	80	80	70	88	62	126	186	70	64	73
k (%/min)		1.1	0.87	0.87	0.99	0.79	1.12	0.55	0.37	0.99	1.08	0.95
Clearance (ml/min/Kg)		2.24	1.51	1.48	1.50	1.50	2.29	0.94	0.75	1.82	2.03	2.28

TABLE 24b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	26.44	
Degrees of Freedom	V1	1	$p < .05$
	V2	50	
Linearity coefficient	F2	0.271	
Degrees of Freedom	V1	3	N.S.
	V2	50	

TABLE 25a. Preoperative gluocse tolerance test
Glucose levels (mmol/1) in non-jaundiced patients

		2	3	4	6	7	8	9	10
TIME (mins)	0	3.51	3.64	3.81	4.00	3.65	4.49	3.30	3.91
	10	11.62	10.72	10.95	14.13	-	10.36	11.03	10.54
	20	10.29	10.60	10.00	13.57	8.50	-	9.05	6.92
	30	9.57	10.16	9.38	12.77	-	10.23	8.36	4.77
	40	8.31	11.44	8.47	8.92	6.81	-	7.16	3.27
	50	9.26	10.99	7.12	7.81	6.94	8.60	6.58	2.60
	60	7.10	9.77	5.86	6.22	4.39	7.56	5.69	3.00
SLOPE (mmol/min)		-.067	-.008	-.105	-.197	-.091	-.088	-.085	-.100
Y-intercept (mmol/1)		12.5	11.2	13.1	20.0	11.7	20.5	11.5	10.5
Half life (min)		72	310	52	35	41	43	58	33
k (%/min)		0.96	0.22	1.33	1.98	1.69	1.61	1.19	2.1
Clearance (ml/min/Kg)		1.85	0.59	2.27	3.13	5.0	1.3	1.72	4.32

TABLE 25b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	16.55	
Degrees of Freedom	V1	1	$p < .05$
	V2	45	
Linearity coefficient	F2	0.118	
Degrees of Freedom	V1	5	N.S.
	V2	45	

Preoperative glucose tolerance test
TABLE 26 Summary of glucose clearance

	Biliary Obstruction (Median)	Control (Median)	
Fasting levels (mmol/l)	4.02 (3.02)	3.66 (1.19)	NS
Slope (mmol/l)	-0.072 (0.069)	-0.09 (0.189)	NS
k (%/min)	0.91 (0.75)	1.47 (1.88)	NS
Half life (min)	73.0 (124.0)	47.5 (277)	NS
Clearance (ml/min/Kg)	1.51 (1.44)	2.06 (4.41)	NS

Figures in this table represent median values of ranges. The figures within brackets denote the extent of each range. Ranges were compared using the Mann Whitney U test. NS denotes no significance for $p > 0.05$.

Preoperative glucose tolerance test

TABLE 27a.

Free fatty acid levels (mmol/l) in patients with obstructive jaundice

		1	2	3	4	5	6	7	8	10	11	12
	0	0.69	0.60	0.70	1.52	0.95	0.59	0.80	0.61	0.44	0.26	0.50
	10	0.60	0.76	0.63	1.15	0.92	0.66	0.76	0.53	0.43	0.16	0.56
	20	0.60	0.70	0.56	0.72	0.86	0.44	0.64	0.41	0.38	0.15	0.66
TIME	30	0.53	0.61	0.72	0.78	0.60	0.31	0.36	0.36	0.32	0.15	0.58
(mins)	40	0.40	0.62	0.46	0.78	0.53	0.25	0.38	0.26	0.26	0.17	0.51
	50	0.28	0.55	0.54	0.49	0.51	0.24	0.38	0.30	0.24	0.21	0.43
	60	0.30	0.63	0.65	0.75	0.46	0.02	0.28	0.20	0.21	0.24	0.46
SLOPE (mmol/min)		-.007	-.001	-.002	-.013	-.009	-.008	-.009	-.007	-.004	0	-.002

TABLE 27b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	19.88	
Degrees of	V1	1	$p < .05$
Freedom	V2	70	
Linearity coefficient	F2	0.296	
Degrees of	V1	5	N.S.
Freedom	V2	70	

Preoperative glucose tolerance test

TABLE 28a. Free fatty acid levels (mmol/l) in non-jaundiced patients

		2	3	4	6	7	8	9	10
	0	0.32	0.84	1.00	0.30	0.36	0.51	0.31	0.49
	10	0.60	1.11	0.74	0.20	0.24	0.55	0.39	0.17
	20	0.39	0.41	0.40	0.18	0.20	0.46	0.27	0.13
TIME	30	0.28	0.12	0.30	0.15	-	0.38	0.24	0.15
(mins)	40	0.24	0.22	0.31	0.15	0.19	0.29	0.18	0.15
	50	0.18	0.15	0.27	0.15	0.14	0.29	0.20	0.16
	60	0.15	0.15	0.41	0.13	0.13	0.23	0.17	0.14
SLOPE (mmol/min)		-.005	-.014	-.010	-.002	-.003	-.005	-.003	-.004

TABLE 28b. Least squares regression analysis for linearity

			Significance
Regression coefficient	F1	26.18	
Degrees of	V1	1	$p < .05$
Freedom	V2	48	
Linearity coefficient	F2	1.02	
Degrees of	V1	5	N.S.
Freedom	V2	48	

TABLE 29a. Preoperative Glucose Tolerance Test

Summary of fasting free fatty acid levels

	MEDIAN (mmol/l)	RANGE (mmol/l)	N	
Bilirubin > 100umol/l	0.610	1.26	11	NS
Bilirubin < 100umol/l	0.365	0.7	8	

TABLE 29b. Preoperative Glucose Tolerance Test

Summary of free fatty acid clearance rates

	MEDIAN (mmol/l)	RANGE (mmol/l)	N	
Bilirubin > 100umol/l	0.007	0.019	11	NS
Bilirubin < 100umol/l	0.005	0.013	8	

Mann Whitney U test

Postoperative alanine tolerance test

TABLE 30a. Alanine levels (mmol/l)

		Jaundiced					Non-jaundiced			
		4	5	8	9	median	6	9	10	median
TIME (mins)	0	0.28	0.25	0.10	0.13	0.19	0.14	0.14	0.09	0.11
	10	1.31	1.65	1.02	1.28		1.40	1.46	1.18	
	20	1.13	1.31	0.96	1.17		1.50	1.37	0.99	
	30	0.98	1.07	0.68	1.07		1.36	1.17	0.86	
	40	0.84	0.82	0.76	0.87		1.26	1.03	0.74	
	50	0.66	0.73	0.61	0.74		0.85	0.89	0.63	
	60	0.62	0.67	0.48	0.72		0.96	0.77	0.54	
Y-intercept (mmol/l)		1.56	1.81	1.23	1.54	1.55	2.01	1.81	1.36	1.81
Slope (mmol/min)		-.010	-.012	-.013	-.017	-.0125	-.016	-.015	-.011	-.015
Half Life (min)		44	38	47	51.5	45.5	49	49	45	47.7
k (%/min)		1.58	1.82	1.47	1.35	1.535	1.41	1.41	1.54	1.453
Clearance (ml/min/Kg)		17.67	17.1	23.52	18.69		21.52	12.52	23.68	

TABLE 30b. Least squares regression analysis for linearity

		Jaundiced		Non-jaundiced	
			Significance		Significance
Regression coefficient	F1	64.41		15.49	
Degrees of	V1	1	$p < .05$	1	$p < .05$
Freedom	V2	21		14	
Linearity coefficient	F2	0.329		0.10	
Degrees of	V1	5	N.S.	5	N.S.
Freedom	V2	21		14	

TABLE 30c

Comparison of preoperative and postoperative alanine clearance in 4 individuals with biliary obstruction (paired data).

	Preoperative		Postoperative			
Fasting alanine	0.11	(0.07)	0.19	(0.18)	n.s.	(mmol/l)
Slope	-0.011	(0.017)	-0.013	(0.007)	n.s.	(mmol/min)
k	1.02	(0.85)	1.53	(0.47)	$p < 0.02$	(%/min)
Half life	69.25	(93)	45.5	(13.5)	$p < 0.02$	(min)
Clearance	12.76	(5.95)	18.18	(6.42)	$p < 0.02$	(ml/min/Kg)

All p values greater than 0.05 are not considered significant. For values less than 0.1 the actual value is listed.

Table 30d

Comparison Of preoperative and postoperative alanine clearance in 3 individuals without biliary obstruction (paired data).

	Preoperative		Postoperative			
Fasting alanine	0.16	(0.05)	0.12	(0.05)	n.s.	(mmol/l)
Slope	-0.011	(0.01)	-0.015	(0.029)	$p < 0.03$	(mmol/min)
k	1.54	(1.71)	1.44	(0.13)	n.s.	(%/min)
Half life	45	(inf)	48	(4)	n.s.	(min)
Clearance	20.22	(20.4)	21.52	(11.16)	$p < 0.06$	(ml/min/Kg)

All p values greater than 0.05 are not considered significant. For values less than 0.1 the actual value is listed.

Postoperative alanine tolerance test

TABLE 31a. Glucose levels (mmol/1)

		Jaundiced				Non-jaundiced		
		4	5	8	9	6	9	10
	0	4.11	4.31	3.64	4.49	3.51	3.53	3.38
	10	4.18	4.42	3.44	4.48	3.65	4.10	3.40
	20	5.92	4.78	3.70	4.68	3.91	3.64	3.60
TIME	30	4.61	5.27	4.06	4.68	4.02	4.30	3.83
(mins)	40	5.57	4.17	4.25	4.89	4.06	4.36	3.74
	50	5.03	5.07	3.88	4.81	4.33	4.26	3.57
	60	5.22	5.04	4.32	4.46	3.73	4.08	3.57
SLOPE (mmol/min)		.017	.010	.012	.003	.009	.009	.004

TABLE 31b. Least squares regression analysis for linearity

		Jaundiced		Non-jaundiced	
			Significance		Significance
Regression coefficient	F1	3.70		5.22	
Degrees of	V1	1	N.S.	1	N.S.
Freedom	V2	21		14	
Linearity coefficient	F2	0.45		1.23	
Degrees of	V1	5	N.S.	5	N.S.
Freedom	V2	21		14	

Postoperative alanine tolerance test

TABLE 32a. Free fatty acid levels (mmol/l)

		Jaundiced				Non-jaundiced		
		4	5	8	9	6	9	10
	0	1.04	0.97	0.71	0.90	0.49	0.38	0.60
	10	0.75	0.90	0.70	0.85	0.40	0.41	0.54
	20	0.58	0.68	0.60	0.66	0.30	0.27	0.25
TIME	30	0.49	0.69	0.50	0.68	0.26	0.24	0.19
(mins)	40	0.35	0.57	0.46	0.53	0.20	0.17	0.39
	50	0.35	0.54	0.53	0.43	0.18	0.18	0.13
	60	0.37	0.51	0.37	0.44	0.16	0.15	0.16
SLOPE (mmol/min)		-.005	-.008	-.011	-.008	-.004	-.007	-.005

TABLE 32b. Least squares regression analysis for linearity

		Jaundiced		Non-jaundiced	
			Significance		Significance
Regression coefficient	F1	79.98		54.05	
Degrees of	V1	1	$p < .05$	1	$p < .05$
Freedom	V2	21		14	
Linearity coefficient	F2	1.17		1.66	
Degrees of	V1	5	N.S.	5	N.S.
Freedom	V2	21		14	

Postoperative alanine tolerance test
TABLE 33a. Lactate levels (mmol/l)

		Jaundiced				Non-jaundiced		
		4	5	8	9	6	9	10
	0	0.96	0.64	1.64	1.02	0.37	0.96	0.88
	10	1.25	0.71	1.54	0.95	0.76	0.89	1.04
	20	1.49	1.23	1.49	0.99	0.59	1.23	1.01
TIME	30	1.24	0.85	1.54	1.19	0.78	1.17	0.89
(mins)	40	1.41	0.89	1.10	0.95	0.74	1.05	0.81
	50	1.15	0.83	1.23	0.84	0.93	0.88	0.68
	60	1.15	0.88	1.59	0.74	0.94	0.90	0.55
SLOPE (mmol/min)		-.004	-.004	.001	.002	-.001	-.007	.008

TABLE 33b. Least squares regression analysis for linearity

		Jaundiced		Non-jaundiced	
			Significance		Significance
Regression coefficient	F1	0.17		0	
Degrees of	V1	1	N.S.	1	N.S.
Freedom	V2	21		14	
Linearity coefficient	F2	0.43		0.42	
Degrees of	V1	5	N.S.	5	N.S.
Freedom	V2	21		14	

Postoperative alanine tolerance test

TABLE 34a. Pyruvate levels (mmol/l)

		Jaundiced				Non-jaundiced		
		4	5	8	9	6	9	10
TIME (mins)	0	0.14	0.07	0.07	0.07	0.09	0.06	0.03
	10	0.22	0.16	0.06	0.12	0.04	0.08	0.06
	20	0.18	0.17	0.06	0.11	0.07	0.07	0.06
	30	0.15	0.17	0.08	0.11	0.07	0.08	0.09
	40	0.17	0.11	0.06	0.10	0.06	0.09	0.08
	50	0.19	0.12	0.10	0.09	0.06	0.09	0.06
	60	0.16	0.10	0.05	0.10	0.06	0.07	0.05
SLOPE (mmol/min)		0	0	0	0	0	0	0

TABLE 34b. Least squares regression analysis for linearity

		Jaundiced		Non-jaundiced	
			Significance		Significance
Regression coefficient	F1	0		0.73	
Degrees of	V1	1	N.S.	1	N.S.
Freedom	V2	21		14	
Linearity coefficient	F2	0.70		0.66	
Degrees of	V1	5	N.S.	5	N.S.
Freedom	V2	21		14	

Postoperative alanine tolerance test

TABLE 35a. Acetoacetate levels (mmol/l)

		Jaundiced				Non-jaundiced		
		4	5	8	9	6	9	10
TIME (mins)	0	0.06	-	-	0.08	-	0.05	0.07
	10	0.07	-	-	0.08	-	0.12	0.11
	20	0.07	-	-	0.11	-	0.13	0.06
	30	0.06	-	-	0.09	-	0.10	0.06
	40	0.06	-	-	0.08	-	0.11	0.06
	50	0.07	-	-	0.06	-	0.11	0.07
	60	0.07	-	-	0.07	-	0.11	0.08
SLOPE (mmol/min)		0	-	-	0	-	.001	0

TABLE 35b. Least squares regression analysis for linearity

		Jaundiced		Non-jaundiced	
			Significance		Significance
Regression coefficient	F1	0.174		0.162	
Degrees of Freedom	V1	1	N.S.	1	N.S.
	V2	7		7	
Linearity coefficient	F2	0.325		0.771	
Degrees of Freedom	V1	5	N.S.	5	N.S.
	V2	7		7	

Postoperative glucose tolerance test

TABLE 36a. Glucose levels (mmol/l)

		Jaundiced				Non-jaundiced	
		4	5	8	11	9	
	0	4.65	4.57	3.33	4.77	4.55	
	10	11.57	9.27	9.28	12.31	10.73	
	20	8.78	9.26	8.30	13.76	9.23	
TIME	30	8.54	8.92	8.18	12.88	8.73	
(mins)	40	7.82	7.22	7.92	10.82	7.55	
	50	7.37	-	7.84	10.33	7.55	
	60	6.92	5.44	7.09	9.69	7.22	
Y-intercept		10.06	12.9	9.08	16.48	10.32	(mmol/l)
Slope		-.049	-.101	-.028	-.107	-.052	(mmol/min)
Half-life		95	52.5	180	72	90	(min)
k		0.73	1.32	0.39	0.96	0.77	(%/min)
Clearance		1.27	1.74	0.83	1.38	1.20	(ml/min/Kg)

TABLE 36b. Least squares regression analysis for linearity

		Jaundiced	
			Significance
Regression coefficient	F1	6.29	
Degrees of	V1	1	$p < .05$
Freedom	V2	20	
Linearity coefficient	F2	0.086	
Degrees of	V1	5	N.S.
Freedom	V2	20	

TABLE 36c

Comparison of preoperative and postoperative glucose clearance in 4 individuals with biliary obstruction (paired data).

	Preoperative		Postoperative			
Fasting alanine	4.54	(2.0)	4.61	(1.46)	n.s.	(mmol/l)
Slope	-0.074	(0.079)	-0.044	(0.069)	n.s.	(mmol/min)
k	0.85	(0.93)	0.96	(0.78)	n.s.	(%/min)
Half life	84	(108)	72.5	(116.5)	n.s.	(min)
Clearance	1.33	(0.91)	1.56	(1.31)	n.s.	(ml/min/Kg)

All p values greater than 0.05 are not considered significant. For values less than 0.1 the actual value is listed.

Postoperative glucose tolerance test

TABLE 37a. FFA levels (mmol/l)

		Jaundiced				Non-jaundiced
		4	5	8	11	9
	0	1.26	0.52	0.72	0.59	0.42
	10	1.00	0.53	0.89	0.58	0.34
	20	0.90	0.46	0.56	0.36	0.38
TIME	30	0.52	0.39	0.66	0.35	0.27
(mins)	40	0.43	0.40	0.65	0.18	0.27
	50	0.42	-	0.59	0.15	0.26
	60	0.45	0.41	0.66	0.14	0.23
SLOPE (mmol/min)		-.015	-.013	-.002	-.009	-.003

TABLE 37b. Least squares regression analysis for linearity

		Jaundiced	
			Significance
Regression coefficient	F1	9.76	
Degrees of	V1	1	$p < .05$
Freedom	V2	20	
Linearity coefficient	F2	0.31	
Degrees of	V1	5	N.S.
Freedom	V2	20	

TABLE 38. Preoperative Anthropometric Measurements

		WT Kg	WT LOSS %	WT/HT %	MAMC %	GS %	LBM Kg
	1	57.7	4.0	115	105	96	39
	2	63.4	13.6	115	91	90	40
	3	70.1	9.0	116	98	92	48
Jaundiced	4	76.0	10.0	109	95	82	55
	5	78.4	8.0	119	115	115	62
Patients	6	62.1	6.0	121	95	112	43
	7	57.5	11.0	87	90	82	48
	8	67.8	17.0	103	95	70	49
	9	66.0	13.0	100	95	75	48
	10	62.2	14.0	114	92	65	40
	11	56.4	4.0	113	98	76	38
	12	54.5	-7.0	118	85	25	37
MEDIAN		62.8	9.3	114.5	95	82	43.5
RANGE		23.9	24	34	30	90	25
	1	60.0	17.0	99	102	62	47
	2	57.2	15.0	94	82	92	48
	3	46.5	18.0	77	75	70	46
Non-jaundiced	4	61.5	15.0	101	91	100	49
	5	58.8	18.0	97	79	35	51
Patients	6	43.5	8.0	92	86	90	33
	7	39.7	0	86	82	80	25
	8	83.3	-1.0	120	101	102	56
	9	83.0	-1.0	112	90	101	65
	10	63.6	13.00	81	89	80	54
MEDIAN		59.4	13.5	94.5	86.5	80.5	48.5
RANGE		21.8	19	43	27	67	40
		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

TABLE 39. Preoperative Biochemical Nutritional Parameters

		ALB	TBK	TFN	TBPA
	1	33	93	1.55	0.07
	2	31	91	2.30	0.12
	3	25	81	-	0.04
Jaundiced	4	33	83	2.20	0.09
	5	41	98	2.55	0.16
Patients	6	34	100	-	0.09
	7	33	91	2.85	0.17
	8	28	81	1.55	0.06
	9	40	89	2.75	0.08
	10	29	104	2.05	0.10
	11	35	90	2.70	0.22
	12	17	-	1.50	0.06
MEDIAN		33	90	3.25	0.09
RANGE		24	23	1.3	0.18
	1	30	-	-	0.13
	2	30	-	3.20	0.07
	3	40	-	2.40	0.17
Non-jaundiced	4	42	-	1.90	0.04
	5	27	-	1.85	0.06
Patients	6	38	-	2.80	0.23
	7	42	-	2.50	0.16
	8	40	-	3.55	0.25
	9	33	-	2.10	0.17
	10	47	-	2.30	0.24
MEDIAN		39	-	2.4	0.165
RANGE		20	-	1.7	0.21
		n.s.	-	n.s.	n.s.

TABLE 40. Preoperative Parameters of Renal Function

		BUN	Se.Cr.	Ur.Urea	CrCl
	1	3.7	74	180	-
	2	3.9	81	135	60.0
	3	7.8	102	375	71.5
Jaundiced	4	8.3	120	435	91.0
	5	4.5	89	498	-
Patients	6	5.0	103	375	62.0
	7	5.8	78	235	63.2
	8	3.9	60	340	133.2
	9	4.1	81	220	49.0
	10	2.0	72	110	120.2
	11	4.7	69	255	88.6
	12	7.1	159	56	12.2
MEDIAN		4.6	81	252	67.4
RANGE		6.3	99	442	121
	1	4.5	126	205	46.3
	2	7.9	126	-	-
	3	13.9	83	-	-
Non-jaundiced	4	4.9	77	-	-
	5	7.0	94	390	66.5
Patients	6	4.2	69	229	120.0
	7	10.2	57	-	-
	8	4.4	91	-	-
	9	7.0	165	-	-
	10	10.1	68	310	173.6
MEDIAN		7.0	87	269.5	93
RANGE		9.7	108	185	108.1
		n.s.	n.s.	n.s.	n.s.

TABLE 41. Preoperative Parameters of Hepatic Function

		Bili	ALP	AST
	1	555	820	91
	2	335	435	140
	3	335	475	48
Jaundiced	4	435	390	95
	5	265	380	42
Patients	6	350	226	57
	7	312	1415	250
	8	165	235	48
	9	400	605	85
	10	255	2470	183
	11	168	153	52
	12	190	2580	325
MEDIAN		323	455	71
RANGE		390	2427	283
	1	6	175	18
	2	8	230	30
	3	12	77	25
Non-jaundiced	4	5	213	32
	5	5	180	17
Patients	6	8	48	37
	7	12	90	30
	8	12	89	32
	9	14	80	28
	10	6	75	13
MEDIAN		8	90	29
RANGE		9	182	24
		$p<.01$	$p<.01$	$p<.01$

TABLE 42. Reference ranges for fasting substrates

Substrate	Fasting range in normal subjects
alanine	not available
glucose	3.5 - 7.8 mmol/l
pyruvate	0.03 - 0.18 mmol/l
acetoacetate	0.03 - 0.11 mmol/l
lactate	0.45 - 1.55 mmol/l
FFA	0.4 - 1.1 mmol/l

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